NAALADASE INHIBITORS FOR TREATING OPIOID TOLERANCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 60/450,690, filed March 3, 2003, the entire contents of which are herein incorporated by reference.

[0002] This invention relates to pharmaceutical compositions and methods for treating opioid tolerance using NAALADase inhibitors.

SUMMARY OF THE INVENTION

[0003] The present invention relates to a method for treating opioid tolerance comprising administering an effective amount of a NAALADase inhibitor to a mammal in need of such treatment.

[0004] The present invention also relates to a pharmaceutical composition comprising:

(i) an effective amount of a NAALADase inhibitor for treating opioid tolerance; and

(ii) a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] FIG. 1 is a graph plotting the time courses of tail-flick responses of mice treated with a placebo, a NAALADase inhibitor, morphine, or a NAALADase inhibitor with morphine.

[0006] FIG. 2 is a bar graph plotting the mean ± S.E.M. Area Under Curve (AUC) values.

DETAILED DESCRIPTION OF THE INVENTION

[0007] Over the last decade, research has provided compelling evidence that glutamate receptors are crucially involved in phenomena related to opioid tolerance [see (Bisaga and Popik 2000) for a recent review]. Glutamate, a major excitatory neurotransmitter in the brain, stimulates both ionotropic and metabotropic glutamate receptors (Monaghan et al. 1989; Conn and Pin 1997). Antagonists of the ionotropic *N*-methyl-D-aspartate (NMDA)

receptor complex including memantine, the low affinity and highly voltage-dependent clinically available NMDA receptor antagonist, inhibit the development of opioid tolerance (Trujillo and Akil 1991; Marek et al. 1991; Popik et al. 2000a) and reverse preexisting tolerance so that opiate-tolerant animals treated with NMDA receptor antagonists become sensitive to doses of morphine that previously did not evoke antinociception (Tiseo and Inturrisi 1993; Popik et al. 2000a).

[0008] Another more "physiological" way of attenuating glutamate neurotransmission could potentially be achieved by inhibiting the metabolism of N-acetyl-aspartyl-glutamate (NAAG) (Slusher et al. 1999), an endogenous dipeptide present in the brain in millimolar (0.5-2.7 mM) concentrations (Pouwels and Frahm 1997) that has been immunohistochemically localized to neurons, particularly those known to be glutamatergic (Williamson and Neale 1988; Tsai et al. 1990; Tsai et al. 1993). NAAG has been hypothesized to be involved in neuronal communication as a neurotransmitter, neuromodulator and precursor of glutamate (Blakely and Coyle 1988). NAAG is released from neurons after depolarization by a calcium-dependent process upon synaptic stimulation (Tsai et al. 1990; Neale et al. 2000), suggesting its neurotransmitter-like properties.

[0009] Importantly, NAAG is hydrolyzed by the neuropeptide glutamate carboxypeptidase (GCP II; EC 3.4.17.21) [N-acetylated-alpha-linked-acidic dipeptidase (NAALADase)] to liberate N-acetyl-aspartate (NAA) and glutamate (Stauch et al. 1989), and the activity of this enzyme can be inhibited by recently developed specific inhibitors (Jackson and Slusher 2001). NAAG itself has been shown to act as a low potency agonist at NMDA receptors (Koenig et al. 1994; Sekiguchi et al. 1992; Westbrook et al. 1986), and thus, according to these data, an inhibition of its metabolism might result in stimulation of NMDA receptors. However, in many other systems it has been shown to antagonize the effects of NMDA receptor activation (Burlina et al. 1994; Grunze et al. 1996; Puttfarcken et al. 1993), and therefore, according to these findings, an inhibition of its metabolism would actually inhibit NMDA receptors. Thus, as noted by Yamamoto et al., (2001a), NAAG acts as an NMDA receptor antagonist at low concentrations but as a low potency NMDA receptor agonist at high concentrations and can therefore be regarded as a mixed agonist / antagonist at the NMDA receptor depending on its concentration (Bruno et al. 1998; Thomas et al. 2000).

[0010] In addition, an increase in NAAG concentration may decrease glutamatergic tone mediated by presynaptic mGluRII receptors (mGluR3), because another line of evidence indicates that NAAG is a direct agonist at mGluRII receptors (Wroblewska et al. 1993; Wroblewska et al. 1997) with EC_{50} ~ 26 uM (Tortella et al. 2000). Other current work demonstrates effects of compounds that inhibit the function of metabotropic receptors on morphine tolerance and reward. Thus, an agonist of group II metabotropic receptors for glutamate (mGluRII), (+)-2-aminobicyclo [3,1,0] hexane-2,6-dicarboxylic acid (LY354740), has been shown to inhibit the development of morphine tolerance (Popik et al. 2000b). Increasing endogenous NAAG concentrations, via inhibition of its hydrolysis via GCPII (NAALADase) might be expected to have similar effects as exogenous addition of an mGluR3 agonist. Lastly, the inhibition of metabolism of NAAG to glutamate via use of a GCPII inhibitor, could directly produce a reduction of extracellular concentration of glutamate, and via this action, may then attenuate the stimulation of both ionotropic and metabotropic receptors for glutamate.

[0011] The pharmacological effects of inhibition of GCP II activity have not been investigated until recently, when specific and potent inhibitors of this enzyme were developed. Among them is 2-phosphonomethyl pentanedioic acid (2-PMPA) (Jackson et al. 1996) that potently inhibits GCP II activity with an inhibition constant (K_i) of 0.3 nM. 2-PMPA is selective for GCP II with no apparent affinity for over 100 different receptors, ion channels, transporters, and enzymes including several glutamatergic sites such as NMDA, AMPA, metabotropic glutamate receptors and glutamate transporters (Slusher et al. 1999).

[0012] Because of its potency and apparent specificity for GCP II, we used 2-PMPA as a prototype compound to explore the role of GCP II inhibition in the development of opiod tolerance.

DEFINITIONS

[0013] "Alkyl" refers to a branched or unbranched saturated hydrocarbon chain comprising a designated number of carbon atoms. For example, C_1-C_9 alkyl is a straight or branched hydrocarbon chain containing 1 to 9 carbon atoms, and includes but is not limited to

substituents such as methyl, ethyl, propyl, iso-propyl, butyl, iso-butyl, tert-butyl, n-pentyl, n-hexyl, and the like, unless otherwise indicated.

[0014] "Alkenyl" refers to a branched or unbranched unsaturated hydrocarbon chain comprising a designated number of carbon atoms. For example, C_2-C_9 alkenyl is a straight or branched hydrocarbon chain containing 2 to 9 carbon atoms having at least one double bond, and includes but is not limited to substituents such as ethenyl, propenyl, iso-propenyl, butenyl, iso-butenyl, tert-butenyl, n-pentenyl, n-hexenyl, and the like, unless otherwise indicated.

[0015] "Alkoxy" refers to the group -OR wherein R is alkyl as herein defined. In one embodiment, R is a branched or unbranched saturated hydrocarbon chain containing 1 to 9 carbon atoms.

[0016] "Carbocycle" refers to a hydrocarbon, cyclic moiety having one or more closed ring(s) that is/are alicyclic, aromatic, fused and/or bridged. Examples include cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane, cyclopentene, cyclohexene, cycloheptene, cycloctene, benzyl, naphthene, anthracene, phenanthracene, biphenyl and pyrene.

[0017] "Aryl" refers to an aromatic, hydrocarbon cyclic moiety having one or more closed ring(s). Examples include, without limitation, phenyl, naphthyl, anthracenyl, phenanthracenyl, biphenyl and pyrenyl.

[0018] "Heterocycle" refers to a cyclic moiety having one or more closed ring(s) that is/are alicyclic, aromatic, fused and/or bridged, with one or more heteroatom(s) (for example, sulfur, nitrogen or oxygen) in at least one of the rings. Examples include, without limitation, pyrrolidine, pyrrole, thiazole, thiophene, piperidine, pyridine, isoxazolidine and isoxazole.

[0019] "Heteroaryl" refers to an aromatic, cyclic moiety having one or more closed ring(s) with one or more heteroatom(s) (for example, sulfur, nitrogen or oxygen) in at least one of the rings. Examples include, without limitation, pyrrole, thiophene, pyridine and isoxazole.

[0020] "Linking group" refers to a moiety that connects the terminal group with the benzene ring in the compounds of formula VI, without compromising with the pharmacological or biological activity of the overall compound.

[0021] "Metal binding group" refers to a functional group capable of interacting with metal ion(s), such as Co^{2+}, Ni^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, or Al^{3+}. Common metal binding groups include amines (e.g. ethylenediamine), aldehydes, ketones, carboxylic acids (e.g. ethylenediaminetetraacetic acid (EDTA)), thiols, phosphorus derivatives and hydroxamic acids.

[0022] "Derivative" refers to a substance produced from another substance either directly or by modification or partial substitution.

[0023] "Effective amount" refers to the amount required to produce the desired effect.

[0024] "Therapeutically effective amount" refers to the amount required to treat glaucoma in an animal or a mammal.

[0025] "Halo" refers to at least one fluoro, chloro, bromo or iodo moiety.

[0026] "Isosteres" refer to elements, functional groups, substitutents, molecules or ions having different molecular formulae but exhibiting similar or identical physical properties. For example, tetrazole is an isostere of carboxylic acid because it mimics the properties of carboxylic acid even though they both have different molecular formulae. Typically, two isosteric molecules have similar or identical volumes and shapes. Ideally, isosteric compounds should be isomorphic and able to co-crystallize. Other physical properties that isosteric compounds usually share include boiling point, density, viscosity and thermal conductivity. However, certain properties are usually different: dipolar moments, polarity, polarization, size and shape since the external orbitals may be hybridized differently. The term "isosteres" encompass "bioisosteres".

[0027] "Bioisosteres" are isosteres that, in addition to their physical similarities, share some common biological properties. Typically, bioisosteres interact with the same recognition site or produce broadly similar biological effects.

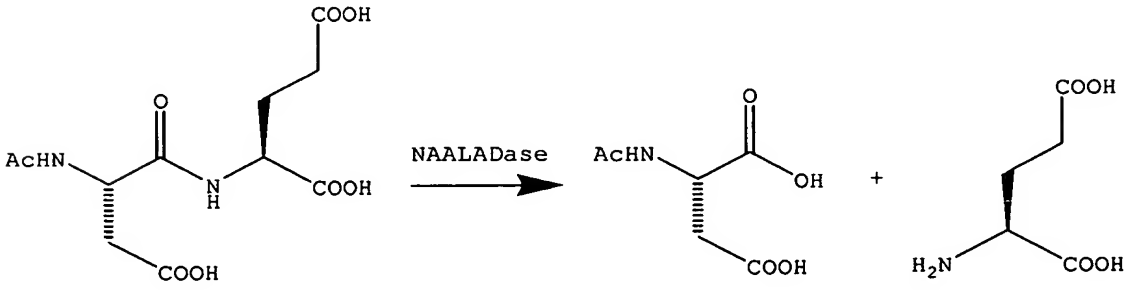
[0028] "Carboxylic acid isosteres" include without limitation direct derivatives such as hydroxamic acids, acyl-cyanamides and acylsulfonamides; planar acidic heterocycles such as tetrazoles, mercaptoazoles, sulfinylazoles, sulfonylazoles, isoxazoles, isothiazoles, hydroxythiadiazoles and hydroxychromes; and nonplanar sulfur- or phosphorus-derived acidic functions such as phosphinates, phosphonates, phosphonamides, sulphonates, sulphonamides, and acylsulphonamides.

[0029] "Metabolite" refers to an intermediate or product resulting from metabolism.

[0030] "NAAG" refers to N-acetyl-aspartyl-glutamate, an important peptide component of the brain, with levels comparable to the major inhibitor neurotransmitter gamma-aminobutyric acid (GABA). NAAG is neuron-specific, present in synaptic vesicles and released upon neuronal stimulation in several systems presumed to be glutamatergic. Studies suggest that NAAG may function as a neurotransmitter and/or neuromodulator in the central nervous system, or as a precursor of the neurotransmitter glutamate. In addition, NAAG is an agonist at group II metabotropic glutamate receptors, specifically mGluR3 receptors; when attached to a moiety capable of inhibiting NAALADase, it is expected that metabotropic glutamate receptor ligands will provide potent and specific NAALADase inhibitors.

[0031] "NAALADase" refers to N-acetylated α-linked acidic dipeptidase, a membrane bound metallopeptidase that catabolizes NAAG to N-acetylaspartate ("NAA") and glutamate ("GLU"):

Catabolism of NAAG by NAALADase



[0032] NAALADase has been assigned to the M28 peptidase family and is also called prostate specific membrane antigen (PSM) or human glutamate carboxypeptidase II (GCP II), EC number 3.4.17.21. It is believed that NAALADase is a co-catalytic zinc/zinc metallopeptidase. NAALADase shows a high affinity for NAAG with a Km of 540 nM. If NAAG is a bioactive peptide, then NAALADase may serve to inactivate NAAG'S synaptic action. Alternatively, if NAAG functions as a precursor for glutamate, the primary function of NAALADase may be to regulate synaptic glutamate availability.

[0033] "Pharmaceutically acceptable carrier" refers to any carrier, diluent, excipient, wetting agent, buffering agent, suspending agent, lubricating agent, adjuvant, vehicle, delivery system, emulsifier, disintegrant, absorbent, preservative, surfactant, colorant, flavorant, or sweetener that would be suitable for use in a pharmaceutical composition. In one embodiment, the pharmaceutically acceptable carrier is non-toxic.

[0034] "Pharmaceutically acceptable equivalent" includes, without limitation, pharmaceutically acceptable salts, hydrates, metabolites, prodrugs, and isosteres. Many pharmaceutically acceptable equivalents are expected to have the same or similar *in vitro* or *in vivo* activity as the inventive compounds.

[0035] "Pharmaceutically acceptable salt" refers to a salt of the inventive compounds that possesses the desired pharmacological activity and that is neither biologically nor otherwise undesirable. The salt can be formed with acids that include without limitation acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycero-phosphate, hemisulfate, heptanoate, hexanoate, hydrochloride hydrobromide, hydroiodide, 2-hydroxyethane-sulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, thiocyanate, tosylate and undecanoate. Examples of a base salt include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine. The basic nitrogen-containing groups can be quarternized with agents including lower alkyl halides such as methyl, ethyl, propyl and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and

diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aralkyl halides such as benzyl and phenethyl bromides.

[0036] "Prodrug" refers to a derivative of the inventive compounds that undergoes biotransformation, such as metabolism, before exhibiting its pharmacological effect(s). The prodrug is formulated with the objective(s) of improved chemical stability, improved patient acceptance and compliance, improved bioavailability, prolonged duration of action, improved organ selectivity, improved formulation (e.g., increased hydrosolubility), and/or decreased side effects (e.g., toxicity). The prodrug can be readily prepared from the inventive compounds using methods known in the art, such as those described by *Burger's Medicinal Chemistry and Drug Chemistry*, Fifth Ed., Vol. 1, pp. 172-178, 949-982 (1995).

[0037] "Inhibition," in the context of enzymes, refers to reversible enzyme inhibition such as competitive, uncompetitive and non-competitive inhibition. Competitive, uncompetitive and non-competitive inhibition can be distinguished by the effects of an inhibitor on the reaction kinetics of an enzyme. Competitive inhibition occurs when the inhibitor combines reversibly with the enzyme in such a way that it competes with a normal substrate for binding at the active site. The affinity between the inhibitor and the enzyme may be measured by the inhibitor constant, K_i, which is defined as:

$$K_i = \frac{[E][I]}{[EI]}$$

wherein [E] is the concentration of the enzyme, [I] is the concentration of the inhibitor, and [EI] is the concentration of the enzyme-inhibitor complex formed by the reaction of the enzyme with the inhibitor. Unless otherwise specified, K_i as used herein refers to the affinity between the inventive compounds and NAALADase. "IC_{50}" is a related term used to define the concentration or amount of a compound that is required to cause a 50% inhibition of the target enzyme.

[0038] "NAALADase inhibitor" refers to any compound that inhibits NAALADase enzyme activity. Embodiments include a NAALADase inhibitor that exhibits a K_i of less than 100

μM, less than 10 μM, and less than 1 μM, as determined using any appropriate assay known in the art.

[0039] "Isomers" refer to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the arrangement or configuration of the atoms.

[0040] "Optical isomers" refer to enantiomers or diastereoisomers.

[0041] "Stereoisomers" are isomers that differ only in the arrangement of the atoms in space.

[0042] "Diastereoisomers" are stereoisomers that are not mirror images of each other. Diastereoisomers occur in compounds having two or more asymmetric carbon atoms; thus, such compounds have 2^n optical isomers, where n is the number of asymmetric carbon atoms

[0043] "Enantiomers" are a pair of stereoisomers that are non-superimposable mirror images of each other. Enantiomers result, for example, from the presence of one or more asymmetric carbon atom(s) in the compound (e.g., glyceraldehyde, lactic acid, sugars, tartaric acid, amino acids).

[0044] "Enantiomer-enriched" refers to a mixture in which one enantiomer predominates.

[0045] "Racemic mixture" means a mixture containing equal amounts of enantiomers.

[0046] "Non-racemic mixture" is a mixture containing unequal amounts of enantiomers.

[0047] "Animal" refers to a living organism having sensation and the power of voluntary movement, and which requires for its existence oxygen and organic food. Examples include, without limitation, members of the human, equine, porcine, bovine, murine, canine, or feline species. In the case of a human, an "animal" may also be referred to as a "patient".

[0048] "Mammal" refers to a warm-blooded vertebrate animal.

[0049] "Opioid" refers to a narcotic analgesic that is either semi or fully synthetic, including, but not limited to Codeine, Morphine, Heroin, Hydromorphone (Dilaudid), Oxycodone (Percodan), Oxymorphone (Numorphan), Hydrocodone (Vicodin), Meperidine (Demerol), Fentanyl, Methadone (Dolophine), Darvon, Talwin.

[0050] "Opioid tolerance" includes without limitation the failure of a steady dose of the drug over time, to sustain the desired pharmacological effect, i.e., the need to increase the drug dosage to maintain the original pharmacological effect.

[0051] "Treating" refers to:

(i) preventing a disease, disorder or condition from occurring in an animal that may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it;

(ii) inhibiting the disease, disorder or condition, i.e., arresting its development; and/or

(iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

[0052] "Treating opioid tolerance" refers to:

(i) preventing opioid tolerance from occurring in an animal that may be predisposed to opioid tolerance but has not yet been diagnosed as having it;

(ii) inhibiting or slowing opioid tolerance, e.g. arresting its development; and/or

(iii) relieving opioid tolerance, e.g. causing its regression.

[0053] One of ordinary skill in the art would recognize that there are alternative nomenclatures, nosologies and classification systems for the diseases, disorders and conditions defined above, and that such systems evolve with medical scientific progress.

[0054] Unless the context clearly dictates otherwise, the definitions of singular terms may be extrapolated to apply to their plural counterparts as they appear in the application; likewise, the definitions of plural terms may be extrapolated to apply to their singular counterparts as they appear in the application.

METHODS OF THE PRESENT INVENTION

[0055] The present invention relates to a method for treating opioid tolerance comprising administering an effective amount of a NAALADase inhibitor to an animal or a mammal in need of such treatment.

PHARMACEUTICAL COMPOSITIONS OF THE PRESENT INVENTION

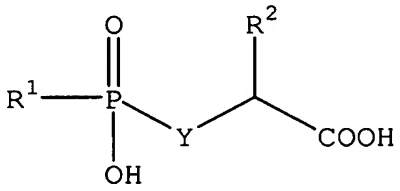
[0056] The present invention further relates to a pharmaceutical composition comprising:

(i) an effective amount of a NAALADase inhibitor for treating opioid tolerance; and

(ii) a pharmaceutically acceptable carrier.

FORMULA I

[0057] An example of a NAALADase inhibitor is a compound of formula I:



I

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

Y is CR^3R^4, NR^5 or O;

R^1 is hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, Ar, $COOR^6$, NR^6R^7 or OR^6, wherein said alkyl, alkenyl, cycloalkyl and cycloalkenyl are independently unsubstituted or substituted with one or more substituent(s) which are, for example, independently selected from carboxy, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, $COOR^6$, NR^6R^7 and Ar;

R^2 is hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, Ar, halo or carboxy, wherein said alkyl, alkenyl, cycloalkyl and cycloalkenyl are

independently unsubstituted or substituted with one or more substituent(s) which are, for example, independently selected from carboxy, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, NR^6R^7 and Ar;

R^3 and R^4 are independently hydrogen or C_1-C_3 alkyl;

R^5 is hydrogen or C_1-C_3 alkyl;

R^6 and R^7 are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl or Ar, wherein said alkyl, alkenyl, cycloalkyl and cycloalkenyl are independently unsubstituted or substituted with one or more substituent(s) which are, for example, independently selected from carboxy, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy and Ar; and

Ar is selected from 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl, wherein said Ar is unsubstituted or substituted with one or more substituent(s) which are, for example, independently selected from halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 alkoxy, C_2-C_6 alkenyloxy, phenoxy, benzyloxy, carboxy and N^6R^7.

[0058] In one embodiment of formula I, Y is CH_2.

[0059] In another embodiment, R^2 is $-(CH_2)_2COOH$.

[0060] In a further embodiment, R^1 is hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, benzyl, phenyl or OR^6, wherein said alkyl, alkenyl, cycloalkyl, cycloalkenyl, benzyl and phenyl are independently unsubstituted or substituted with one or more substituent(s) independently selected from carboxy, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 alkoxy, C_2-C_6 alkenyloxy, phenoxy, benzyloxy, NR^6R^7, benzyl and phenyl.

[0061] Examples of compounds of formula I include without limitation:

2-(phosphonomethyl)pentanedioic acid;

2-[[(2-carboxyethyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[(benzylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[(phenylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[[((hydroxy)phenylmethyl)hydroxyphosphinyl]-methyl]pentanedioic acid;

2-[(butylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[[(3-methylbenzyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[(3-phenylpropylhydroxyphosphinyl)methyl]-pentanedioic acid;

2-[[(4-fluorophenyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[(methylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[(phenylethylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[[(4-methylbenzyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[[(4-fluorobenzyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[[(4-methoxybenzyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[[(3-trifluoromethylbenzyl)hydroxyphosphinyl]-methyl]pentanedioic acid;

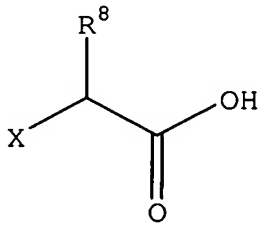
2-[[4-trifluoromethylbenzyl)hydroxyphosphinyl]-methyl]pentanedioic acid;

2-[[(2-fluorobenzyl)hydroxyphosphinyl]methyl]-pentanedioic acid;

2-[[(2,3,4,5,6-pentafluorobenzyl)hydroxy-phosphinyl]methyl]pentanedioic acid; and enantiomers and pharmaceutically acceptable equivalents.

FORMULA II

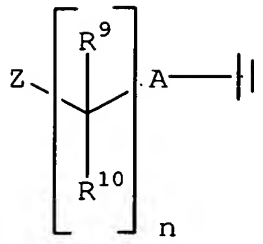
[0062] Another NAALADase inhibitor is a compound of formula II



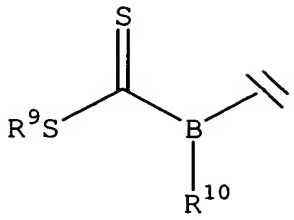
II

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

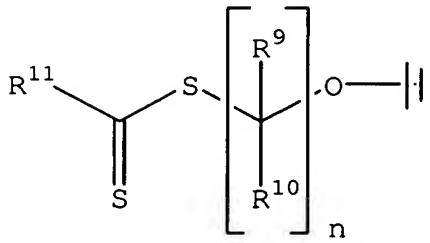
X is a moiety of formula III, IV or V



III



IV



V ;

Z is SH, SO_{3H}, SO_2H, SOH, $SO(NH)R^{12}$ or $S(NHR^{12})_2R^{13}$;

B is N or CR^{14};

A is O, S, $CR^{15}R^{16}$ or $(CR^{15}R^{16})_mS$;

m and n are independently 0, 1, 2, 3 or 4;

R^8, R^9, R^{10}, R^{11}, R^{12}, R^{14}, R^{15} and R^{16} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, Ar^1, hydroxy, carboxy, carbonyl, amino, cyano, isocyano, nitro, sulfonyl, sulfoxy, thio, thiocarbonyl, thiocyano, formanilido, thioformamido, sulfhydryl, halo, haloalkyl, trifluoromethyl or oxy, wherein said alkyl, alkenyl, cycloalkyl and cycloalkenyl are independently unsubstituted or substituted with one or more substituent(s); and

Ar^1 is a carbocyclic or heterocyclic moiety, which is unsubstituted or substituted with one or more substituent(s);

provided that when X is a moiety of formula III and A is O, then n is 2, 3 or 4; when X is a moiety of formula III and A is S, then n is 2, 3 or 4; and when X is a moiety of formula III and A is $(CR^{15}R^{16})_mS$, then n is 0, 2, 3 or 4.

[0063] In one embodiment of formula II, X is a moiety of formula III; n is 0, 1, 2 or 3; Z is SH, SO_3H, SO_2H, SOH or $S(NHR^{12})_2R^{13}$; and A is O, S or $CR^{15}R^{16}$.

[0064] In another embodiment, R^8 is $-(CH_2)_2COOH$.

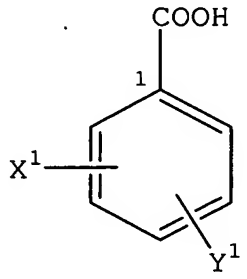
[0065] In a further embodiment, Z is SH.

[0066] Examples of compounds of formula II include without limitation:

2-(2-sulfanylethyl)pentanedioic acid;
3-(2-sulfanylethyl)-1,3,5-pentanetricarboxylic acid;
2-(2-sulfanylpropyl)pentanedioic acid;
2-(2-sulfanylbutyl)pentanedioic acid;
2-(2-sulfanyl-2-phenylethyl)pentanedioic acid;
2-(2-sulfanylhexyl)pentanedioic acid;
2-(2-sulfanyl-1-methylethyl)pentanedioic acid;
2-[1-(sulfanylmethyl)propyl]pentanedioic acid;
2-(3-sulfanylpentyl)pentanedioic acid;
2-(3-sulfanylpropyl)pentanedioic acid;
2-(3-sulfanyl-2-methylpropyl)pentanedioic acid;
2-(3-sulfanyl-2-phenylpropyl)pentanedioic acid;
2-(3-sulfanylbutyl)pentanedioic acid;
2-[3-sulfanyl-2-(phenylmethyl)propyl]pentanedioic acid;
2-[2-(sulfanylmethyl)butyl]pentanedioic acid;
2-[2-(sulfanylmethyl)pentyl]pentanedioic acid;
2-(3-sulfanyl-4-methylpentyl)pentanedioic acid; and
enantiomers and pharmaceutically acceptable equivalents.

FORMULA VI

[0067] Another NAALADase inhibitor is a compound of formula VI



VI

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

X^1 is -W-Z^1;

W is a bond or a linking group;

Z^1 is a terminal group; and

Y^1 is -COOH oriented *meta* or *para* relative to C-1.

[0068] Linking groups include, without limitation, divalent hydrocarbon chains, ethers, sulfides and amines, wherein the hydrocarbon chain, whether alone or part of the ether, sulfide or amine, may be saturated or unsaturated, straight or branched, open or closed, unsubstituted or substituted with one or more substituent(s) which are, for example, independently selected from C_1-C_6 alkoxy, C_2-C_6 alkenyloxy, phenoxy, benzyloxy, hydroxy, carboxy, carbamido, carbamoyl, carbamyl, carbonyl, carbozoyl, amino, hydroxyamino, formamido, formyl, guanyl, cyano, cyanoamino, isocyano, isocyanato, diazo, azido, hydrazino, triazano, nitro, nitroso, isonitroso, nitrosamino, imino, nitrilo, isonitrilo, nitrosimino, oxo, C_1-C_6 alkylthio, sulfamino, sulfamoyl, sulfeno, sulfhydryl, sulfinyl, sulfo, sulfonyl, sulfoxy, thiocarboxy, thiocyano, isothiocyano, thioformamido, halo, haloalkyl, chlorosyl, chloryl, perchloryl, trifluoromethyl, iodosyl, iodyl, phosphino, phosphinyl, phospho, phosphono, arsino, selanyl, diselanyl, siloxy, silyl and silylene.

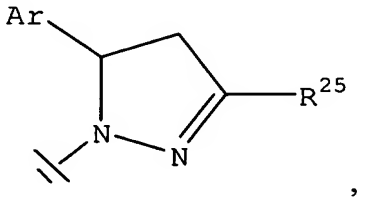
[0069] W is a bond, -$(CR^{17}R^{18})_n$-, -$(CR^{17}R^{18})_nO(CR^{19}R^{20})_m$-, -$(CR^{17}R^{18})_nS(CR^{19}R^{20})_m$- or -$(CR^{17}R^{18})_nNR^{21}(CR^{19}R^{20})_m$-, wherein m and n are independently 0-9, and R^{17}, R^{18}, R^{19}, R^{20} and R^{21} are independently hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_6-C_{14} aryl, heteroaryl, C_6-C_{14} carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino or C_1-C_6 alkoxy, and said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle and alkoxy

are independently unsubstituted or substituted with one or more substituent(s). In one embodiment, R^{17}, R^{18}, R^{19}, R^{20} and R^{21} are each hydrogen and the total number of carbon atoms in W is 2-6.

[0070] Z^1 is a metal binding group. In one embodiment, Z^1 is -COOH, $-COR^{22}$, $-OR^{22}$, $-CF_3$, -CN, -F, -Cl, -Br, -I, -NO, $-NO_2$, $-C(O)(NR^{22}OR^{23})$, $-C(O)(NR^{22}PO_3H_2)$, $-C(O)(NR^{22}R^{23})$, =NOH, $-NR^{22}(P(O)(R^{23})OH)$, $=NR^{22}$, $-N=NR^{22}$, $-N(R^{22})CN$, $-NR^{22}(CR^{23}R^{24})_pCOOH$, $-NR^{22}(CO)NR^{23}R^{24}$, $-NR^{22}(COOR^{23})$, $-NR^{22}(CO)R^{23}$, $-NR^{22}(OR^{23})$, $-NR^{22}R^{23}$, $-NR^{22}(SO_2R^{23})$, $-O(CO)R^{22}$, $-OR^{22}$, $-SO_2(OR^{22})$, $-SO_2(NR^{22}R^{23})$, $-SO_2R^{22}$, $-SO_3R^{22}$, $-SNR^{22}(OR^{23})$, $-S(NR^{22}R^{23})$, $-SR^{22}$, $-SSR^{22}$, $-P(O)(OH)OR^{22}$, $-P(O)(OH)R^{22}$ or $-PR^{22}R^{23}$, wherein p is 0-6, and R^{22}, R^{23} and R^{24} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_6-C_{14} aryl, heteroaryl, C_6-C_{14} carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino or C_1-C_9 alkoxy, and said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle and alkoxy are independently unsubstituted or substituted with one or more substituent(s). In another embodiment, Z^1 is $-NH(CR^{23}R^{24})_pCOOH$, $-PO(OH)OR^{22}$, $-PO(OH)R^{22}$, $-NR^{22}(P(O)(R^{23})OH)$, $-CON(R^{22})(OH)$ or -SH.

[0071] In one embodiment of formula VI:

X^1 is $-(CR^{17}R^{18})_nNH(CR^{19}R^{20})_mCOOH$, $-PO(OH)OR^{22}$, $-(CR^{17}R^{18})_nP(O)(OH)R^{22}$, $-NH-(CR^{19}R^{20})_m$-heteroaryl, $-NH(P(O)(R^{23})OH)$, $-(CR^{17}R^{18})_nNH(P(O)(OH)R^{23})$, $-CON(R^{22})(OH)$ $-(CR^{17}CR^{18})_nCON(R^{22})(OH)$, $-(CR^{17}R^{18})_nSH$ or $-O(CR^{19}R^{20})_mSH$, $-SO_2NH$-aryl, $-N(C=O)-CH_2(C=O)$-aryl, $-SO_2NH$-aryl, $-N(C=O)-CH_2(C=O)$-aryl, -O-aryl wherein aryl in -O-aryl is substituted by at least one of nitro, carboxy or



,

wherein X^1 is oriented *meta* or *para* relative to C-1;

m and n are independently 1-3, provided that when X^1 is $-O(CR^{19}R^{20})_mSH$, then m is

2 or 3;

R^{17}, R^{18}, R^{19}, R^{20}, R^{22}, R^{23} and R^{25} are independently hydrogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino or C_1-C_6 alkoxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle and alkoxy are independently unsubstituted or substituted with one or more substituent(s); and

Y^1 is -COOH oriented *meta* or *para* relative to C-1.

[0072] When X is $-PO(OH)OR^{22}$ or $-(CR^{17}R^{18})_nP(O)(OH)OR^{22}$, then R^{22} is not H or methyl; when X is $-NH(P(O)(R^{23})OH$ or $-(CR^{17}R^{18})_nNH(P(O)(OH)R^{23})$, then R^{23} is not benzyl unsubstituted or substituted with amino; and when X is $-CON(R^{22})(OH)$, then R^{22} is not H or methyl.

[0073] In another embodiment of formula VI, X^1 is oriented *meta* relative to C-1, and Y^1 is oriented *ortho* relative to X^1 and *para* relative to C-1. In a further embodiment, W is a bond, $-(CH_2)_n-NH-(CH_2)_m-$ or $-(CH_2)_n-$; m is 1-3; n is 0-3; and Z^1 is $-CO_2H$, $-NO_2$, $-NH_2$, $-SO_3H$, halo, C_5-C_6 heteroaryl, carboxyphenylthio, or mono- or di-carboxyphenylsulfonyl.

[0074] Examples of this embodiment include:

2-[(4-carboxyphenyl)sulfonyl]-1,4-benzene-dicarboxylic acid;

2-[(2,5-dicarboxyphenyl)sulfonyl]-1,4-benzene-dicarboxylic acid;

1,2,4-benzenetricarboxylic acid;

2-[(2-carboxyphenyl)thio]-1,4-benzenedicarboxylic acid;

2-nitro-1,4-benzenedicarboxylic acid;

2-bromo-1,4-benzenedicarboxylic acid;

2-amino-1,4-benzenedicarboxylic acid;

2-sulfoterephthalic acid, monosodium salt;

2-carboxymethyl-1,4-benzenedicarboxylic acid;

2-[(2-furanylmethyl)-amino]-1,4-benzenedicarboxylic acid;

2-[(carboxymethyl)amino]-1,4-benzenedicarboxylic acid; and

enantiomers and pharmaceutically acceptable equivalents.

[0075] In another embodiment of formula VI, X^1 is oriented *ortho* relative to C-1, and Y^1 is oriented *para* relative to X^1 and *meta* relative to C-1. In one embodiment, (1) when W is a bond, then Z^1 is $-CO_2H$, -OH, $-NO_2$, $-C(O)(NHR^{23})$, $-SR^{23}$, $-COR^{23}$ or $-NH(CH_2R^{23})$, and R^{23} is an aryl or a heteroaryl wherein said aryl and heteroaryl are independently unsubstituted or substituted with one or more alkyl, nitro or carboxy group(s); and (2) when W is $-(CH_2)_n-$ and n is 1-3, then Z^1 is –SH.

[0076] Examples of this embodiment include:

4-(4-nitrobenzoyl)-1,3-benzenedicarboxylic acid;

4-[4-(2,4-dicarboxybenzoyl)phenoxy]-1,2-benzene-dicarboxylic acid;

4-[[(2,4,6-trimethylphenyl)amino]carbonyl]-1,3-benzenedicarboxylic acid;

4-nitro-1,3-benzenedicarboxylic acid;

4-[(1-naphthalenylamino)-carbonyl]-1,3-benzene-dicarboxylic acid;

1,2,4-benzenetricarboxylic acid;

4-[(2-carboxyphenyl)thio]-1,3-benzenedicarboxylic acid;

4-[3-[[3-(2,4-dicarboxyphenoxy)propyl]dithio]-propoxy]-1,3-benzenedicarboxylic acid;

4-hydroxy-1,3-benzenedicarboxylic acid;

4-[(2-furanylmethyl)amino]-1,3-benzenedicarboxylic acid;

4-(2-mercaptoethyl)-1,3-benzenedicarboxylic acid; and

enantiomers and pharmaceutically acceptable equivalents.

[0077] In another embodiment of formula VI, X^1 is oriented *meta* relative to C-1, and Y^1 is oriented *meta* relative to X^1 and *meta* relative to C-1. In one embodiment, (1) when W is a bond, $-(CH_2)_n-$ or $-O(CH_2)_m-$ and m and n are independently 0-3, then Z^1 is $-SO_3H$, $-NO_2$, $-NH_2$, $-CO_2H$, -OH, $-PO_3H$, -CO(NHOH) or –SH; (2) when W is $-(CH_2)_nNH(CH_2)_m-$ and m and n are independently 0-3, then Z^1 is $-CO_2H$ or C_5-C_6 heteroaryl; and (3) when W is a bond, then Z^1 is either (a) a heteroaryl that is unsubstituted or substituted with an aryl that is unsubstituted or substituted with one or more C_1-C_3 alkyl, halo, nitro or hydroxy group(s), or (b) $-SO_2(NHR^{24})$ or $-NH(COR^{24})$, wherein R^{24} is an aryl that is unsubstituted or substituted with one or more nitro, amino, halo or hydroxy group(s).

[0078] Examples of this embodiment include:

5-[4,5-dihydro-5-(4-hydroxyphenyl)-3-phenyl-1H-pyrazol-1-yl]-1,3-benzenedicarboxylic acid;

5-(4,5-dihydro-3-methyl-5-phenyl-1H-pyrazol-1-yl)-1,3-benzenedicarboxylic acid;

5-[[(4-chloro-3-nitrophenyl)amino]sulfonyl]-1,3-benzenedicarboxylic acid;

5-[[[4-chloro-3-[[3-(2-methoxyphenyl)-1,3-dioxopropyl]amino]phenyl]amino]sulfonyl-1,3-benzenedicarboxylic acid;

5-[[3-[4-(acetylamino)phenyl]-1,3-dioxopropyl]amino]-1,3-benzenedicarboxylic acid;

5-acetylamino-1,3-benzenedicarboxylic acid;

5-[[(1-hydroxy-2-naphthalenyl)carbonyl]-methylamino]-1,3-benzenedicarboxylic acid;

5-(4-carboxy-2-nitrophenoxy)-1,3-benzenedicarboxylic acid;

5-sulfo-1,3-benzenedicarboxylic acid;

5-nitro-1,3-benzenedicarboxylic acid;

5-amino-1,3-benzenedicarboxylic acid;

1,3,5-benzenetricarboxylic acid;

5-[[(3-amino-4-chlorophenyl)amino]sulfonyl]-1,3-benzenedicarboxylic acid;

5-(3-mercaptopropoxy)-1,3-benzenedicarboxylic acid;

5-hydroxy-1,3-benzenedicarboxylic acid;

5-(2-mercaptoethoxy)-1,3-benzenedicarboxylic acid;

5-[(hydroxyamino)carbonyl]-1,3-benzenedicarboxylic acid;

5-phosphono-1,3-benzenedicarboxylic acid;

5-mercaptomethyl-1,3-benzenedicarboxylic acid;

5-phosphonomethyl-1,3-benzenedicarboxylic acid;

5-[[(carboxymethyl)amino]-methyl]-1,3-benzene-dicarboxylic acid;

5-[(carboxymethyl)amino]-1,3-benzenedicarboxylic acid;

5-[[(2-furanylmethyl)amino]-methyl]-1,3-benzene-dicarboxylic acid;

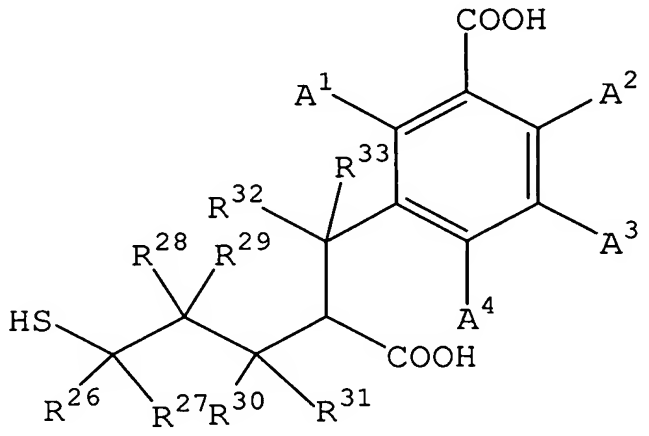
5-[2-(hydroxyamino)-2-oxoethyl]-1,3-benzene-dicarboxylic acid;

5-(2-mercaptoethyl)-1,3-benzenedicarboxylic acid; and

enantiomers and pharmaceutically acceptable equivalents.

FORMULA VII

[0079] Another NAALADase inhibitor is a compound of formula VII



VII

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32} and R^{33} are independently hydrogen or C_1-C_3 alkyl;

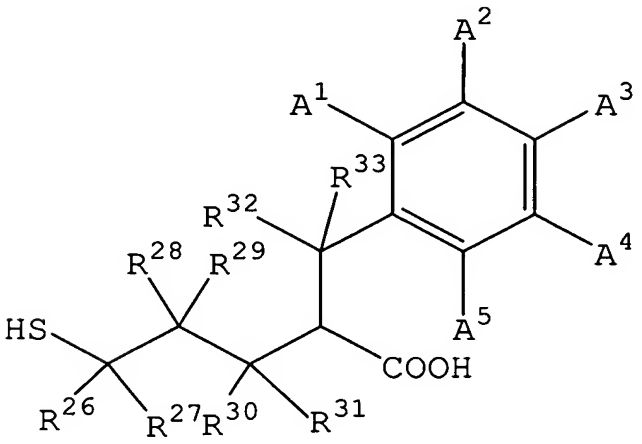
A^1, A^2, A^3 and A^4 are independently hydrogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, halo, nitro, phenyl, phenoxy, benzyl, benzyloxy or -COOH, or any adjacent two of A^2, A^3 and A^4 form with the benzene ring a fused 5- or 6-membered carbocyclic or heterocyclic aromatic ring, said heterocyclic aromatic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s).

[0080] In one embodiment, R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32} and R^{33} are independently hydrogen or methyl; and A^1, A^2, A^3 and A^4 are independently hydrogen, C_1-C_4 alkyl, C_1-C_2 alkoxy, halo, nitro, phenyl, phenoxy, benzyloxy, nitro or -COOH.

[0081] In another embodiment, any adjacent two of A^2, A^3 and A^4 form with the benzene ring a fused 5- or 6-membered carbocyclic or heterocyclic aromatic ring, said heterocyclic aromatic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s).

FORMULA VIII

[0082] Another NAALADase inhibitor is a compound of formula VIII



VIII

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32} and R^{33} are independently hydrogen or C_1-C_3 alkyl; and

A^1, A^2, A^3, A^4 and A^5 are independently hydrogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_3 perhaloalkyl, phenyl, phenoxy, benzyl, benzyloxy, hydroxy, halo, cyano, nitro, $-SO_2R^{34}$, $-(C=O)NR^{34}R^{35}$, $-(C=O)NR^{34}(CH_2)_nCOOH$, $-NR^{34}(C=O)R^{35}$, $-(CH_2)_nCOOH$ or –COOH, or any adjacent two of A^1, A^2, A^3, A^4 and A^5 form with the benzene ring a fused 5- or 6-membered carbocyclic or heterocyclic aromatic ring, said heterocyclic aromatic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s);

R^{34} and R^{35} are independently hydrogen, C_1-C_6 alkyl, phenyl or benzyl; and

n is 1-3.

[0083] If A^1, A^3 and A^5 are independently hydrogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, halo, nitro, phenyl, phenoxy, benzyl, benzyloxy or -COOH, then neither A^2 nor A^4 are –COOH; and if any adjacent two of A^3, A^4 and A^5 form with the benzene ring a fused 5- or 6-membered carbocyclic or heterocyclic aromatic ring, said heterocyclic aromatic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s), then A^2 is not –COOH.

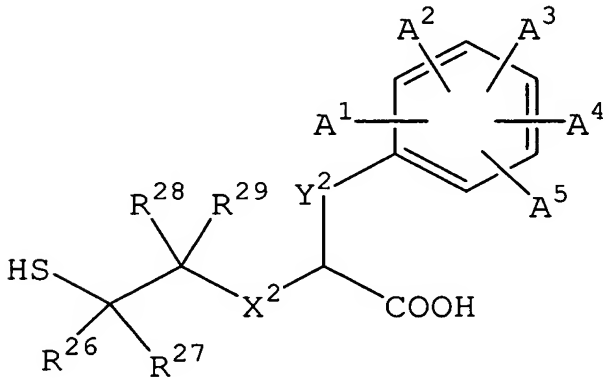
[0084] In one embodiment, R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32} and R^{33} are each hydrogen; A^1, A^2, A^3, A^4 and A^5 are independently hydrogen, C_1-C_4 alkyl, C_1-C_2 alkoxy, C_1-C_2 perhaloalkyl, phenyl, phenoxy, hydroxy, halo, cyano, nitro, $-SO_2R^{34}$, $-(C=O)NR^{34}R^{35}$,

-(C=O)NR^{34}(CH$_2$)COOH, -NR^{34}(C=O)R^{35} or -(CH$_2$)COOH; and R^{34} and R^{35} are independently hydrogen, methyl or benzyl.

[0085] In another embodiment, any adjacent two of A^1, A^2, A^3, A^4 and A^5 form with the benzene ring a fused 5- or 6-membered carbocyclic or heterocyclic aromatic ring, said heterocyclic aromatic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s).

FORMULA IX

[0086] Another NAALADase inhibitor is a compound of formula IX



IX

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

X^2 and Y^2 are independently –CR^{30}R^{31}-, -O-, -S- or -NR^{30}-, provided that at least one of X^2 and Y^2 is/are -CR^{30}R^{31}-;

A^1, A^2, A^3, A^4 and A^5 are independently hydrogen, C$_1$-C$_9$ alkyl, C$_2$-C$_9$ alkenyl, C$_2$-C$_9$ alkynyl, aryl, heteroaryl, carbocycle, heterocycle, C$_1$-C$_9$ alkoxy, C$_2$-C$_9$ alkenyloxy, phenoxy, benzyloxy, hydroxy, halo, nitro, cyano, isocyano, -COOR^{34}, -COR^{34}, -NR^{34}R^{35}, -SR^{34}, -SOR^{34}, -SO$_2$R^{34}, -SO$_2$(OR^{34}), -(C=O)NR^{34}R^{35}, -(C=O)NR^{34}(CH$_2$)$_n$COOH, -NR^{34}(C=O)R^{35} or -(CH$_2$)$_n$COOH, or any adjacent two of A^1, A^2, A^3, A^4 and A^5 form with the benzene ring a fused ring that is saturated or unsaturated, aromatic or non-aromatic, and carbocyclic or heterocyclic, said heterocyclic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s);

n is 1-3; and

R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{34} and R^{35} are independently hydrogen, C$_1$-C$_9$ alkyl, C$_2$-C$_9$ alkenyl, C$_2$-C$_9$ alkynyl, aryl, heteroaryl, carbocycle or heterocycle; and said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy,

benzyloxy, and fused ring are independently unsubstituted or substituted with one or more substituent(s).

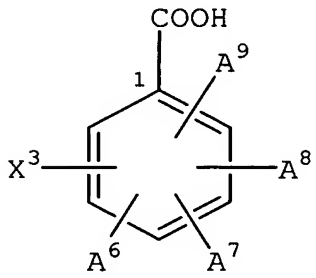
[0087] If A^1, A^2 and A^3 are each hydrogen, and A^4 and A^5 are each –COOH, then A^4 is *ortho* to A^5; and if Y^3 is $-CR^{30}R^{31}-$, then at least one of A^1, A^2, A^3, A^4 and A^5 is/are independently phenoxy, benzyloxy, aryl, heteroaryl, carbocycle or heterocycle that is substituted with one or more substituent(s).

[0088] In one embodiment, Y^2 is –O-, -S- or $-NR^{30}-$; A^1, A^2, A^3, A^4 and A^5 are independently hydrogen, C_1-C_4 alkyl, C_1-C_2 alkoxy, hydroxy, halo, -COOH, $-COR^{34}$, $-NR^{34}(C=O)R^{35}$ or $-(CH_2)COOH$; and R^{34} and R^{35} are independently hydrogen or methyl.

[0089] In another embodiment, Y^2 is $-CR^{30}R^{31}-$; A^1, A^2, A^3 and A^4 are each hydrogen; and A^5 is phenoxy, benzyloxy, aryl, heteroaryl, carbocycle or heterocycle, wherein said phenoxy and benzyloxy are substituted with –COOH, and said aryl, heteroaryl, carbocycle and heterocycle are independently substituted with one or more substituent(s) selected from cyano and –COOH.

FORMULA X

[0090] Another NAALADase inhibitor is a compound of formula X



X

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

X^3 is $-(CR^{36}R^{37})_nSH$, $-O(CR^{36}R^{37})_2SH$, $-S(CR^{36}R^{37})_2SH$ or $-NR(CR^{36}R^{37})_2SH$;

n is 1-3; and

R, R^{36}, R^{37}, A^6, A^7, A^8 and A^9 are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkylsulfonyl, C_1-C_9 alkoxy, C_2-C_9 alkenoxy,

phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenoxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s).

[0091] Examples of compounds of formula X include without limitation:

3-(2-mercaptoethyl)-benzoic acid;
3-(mercaptomethyl)-benzoic acid;
2-(mercaptomethyl)-benzoic acid;
5-hydroxy-2-(2-mercaptoethyl)-benzoic acid;
2-(2-mercaptoethyl)-benzoic acid;
5-[(4-carboxyphenyl)methoxy]-2-(2-mercaptoethyl)-benzoic acid;
2-(2-mercaptoethyl)-5-(phenylmethoxy)-benzoic acid;
2-(carboxymethoxy)-6-(2-mercaptoethyl)-benzoic acid;
5-[(3-carboxyphenyl)methoxy]-2-(2-mercaptoethyl)-benzoic acid;
2-(2-mercaptoethyl)-6-(phenylmethoxy)-benzoic acid;
2-[(2-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;
2-[(4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;
3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid;
2-(3,3-dimethylbutoxy)-6-(2-mercaptoethyl)-benzoic acid;
2-(2-mercaptoethyl)-6-(2-phenylethoxy)-benzoic acid;
2-[(2-chlorophenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;
2-[[3-carboxy-5-(1,1-dimethylethyl)phenyl]methoxy]-6-(2-mercaptoethyl)-benzoic acid;
2-(2-mercaptoethyl)-6-phenoxy-benzoic acid;
2-(2-mercaptoethyl)-6-phenylamino-benzoic acid;
2-(2-mercaptoethyl)-6-(phenylthio)-benzoic acid;
5'-(1,1-dimethylethyl)-3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid;
3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,4'-dicarboxylic acid;
2-[(4-carboxy-2-methoxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;
2-[(4-carboxy-3-methoxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;
2-[(2-bromo-4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;
2-[(3-bromo-4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[(4-chlorophenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-(biphenyl-2-ylmethoxy)-6-(2-mercaptoethyl)-benzoic acid;

2-[(3-bromo-5-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[(2-bromo-5-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-(2-mercaptoethyl)-6-[(4-methoxyphenyl)methoxy]-benzoic acid;

2-(2-mercaptoethyl)-6-[(4-methylphenyl)methoxy]-benzoic acid;

2-[(4-bromo-3-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

2-[(2-carboxy-5-methoxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid;

5-(mercaptomethyl)-2-(2-phenylethoxy)-benzoic acid;

2-bromo-5-(mercaptomethyl)-benzoic acid;

4-(mercaptomethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid;

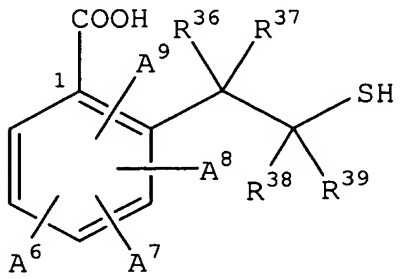
5-(mercaptomethyl)-2-(phenylmethoxy)-benzoic acid; and

4-bromo-3-(mercaptomethyl)-benzoic acid; and

enantiomers and pharmaceutically acceptable equivalents.

FORMULA XI

[0092] Another NAALADase inhibitor is a compound of formula XI



XI

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

R^{37}, R^{38}, R^{39} and R^{40} are independently hydrogen or C_1-C_3 alkyl; and

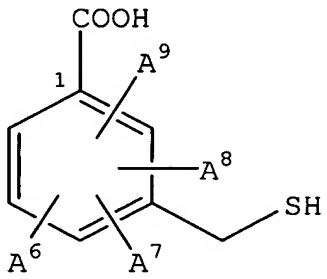
A^6, A^7, A^8 and A^9 are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkylsulfonyl, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenoxy,

phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s).

[0093] In one embodiment, R^{36}, R^{37}, R^{38} and R^{39}, A^7, A^8 and A^9 are each hydrogen; A^6 is hydrogen, $-(CH_2)_n-W^1$, or $-Y^3-(CH_2)_n-W^1$; n is 0-3; Y^3 is O, S or NR^{40}; R^{40} is hydrogen or C_1-C_4 alkyl; W^1 is C_1-C_6 alkyl or phenyl, wherein W^1 is unsubstituted or substituted with C_1-C_4 alkyl, C_1-C_4 alkoxy, carboxy or halo.

FORMULA XII

[0094] Another NAALADase inhibitor is a compound of formula XII



XII

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

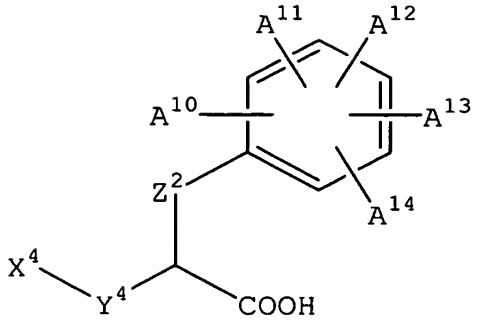
A^6, A^7, A^8 and A^9 are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkylsulfonyl, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenoxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s).

[0095] At least one of A^6, A^7, A^8 and A^9 is/are not hydrogen; and if A^6 is chloro, fluoro, amino or thiomethyl, then at least one of A^7, A^8 and A^9 is/are not hydrogen.

[0096] In one embodiment, A^7, A^8 and A^9 are each hydrogen; A^6 is $-(CH_2)_n-Ar^2$ or $-Y^3-(CH_2)_n-Ar^2$; n is 0-3; Y^3 is O, S or NR^{41}; R^{41} is hydrogen or C_1-C_4 alkyl; and Ar^2 is phenyl, wherein Ar^2 is unsubstituted or substituted with C_1-C_4 alkyl, carboxy or halo.

FORMULA XIII

[0097] Another NAALADase inhibitor is a compound of formula XIII



XIII

or an enantiomer pharmaceutically acceptable equivalent of said compound, wherein:

X^4 is -(CO)NHOH or -N(OH)COH;

Y^4 is a bond or a divalent linking group having from 1 to 9 carbon atom(s) and from 0 to 5 heteroatom(s) independently selected from oxygen, sulfur and nitrogen;

Z^2 is $-CR^{41}R^{42}-$, $-NR^{41}-$, -O- or -S-;

A^{10}, A^{11}, A^{12}, A^{13} and A^{14} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, hydroxy, halo, nitro, cyano, isocyano, $-COOR^{43}$, $-COR^{43}$, $-NR^{43}R^{44}$, $-SR^{43}$, $-SOR^{43}$, $-SO_2R^{43}$, $-SO_2(OR^{43})$, $-(CO)NR^{43}R^{43}$, $-(CO)NR^{43}(CH_2)_nCOOH$, $-NR^{43}(CO)R^{44}$ or $-(CH_2)_nCOOH$, or any adjacent two of A^{10}, A^{11}, A^{12} and A^{13} form with the benzene ring a fused ring that is saturated or unsaturated, aromatic or non-aromatic, and carbocyclic or heterocyclic, said heterocyclic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s);

n is 1-3;

R^{41}, R^{42}, R^{43} and R^{44} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle or heterocycle; and

said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy, benzyloxy, and fused ring are independently unsubstituted or substituted with one or more substituent(s).

[0098] In one embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$, $-NR^{49}-$, -O-, -S- or $-SO_2-$; p and q are independently 0-4, provided that when q is 0 and W^2 is $-NR^{49}-$, -O-, -S- or $-SO_2-$, then Z^2 is $-CR^{41}R^{42}-$; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkoxy, C_2-C_9 alkenoxy,

phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s); and A^{10}, A^{11} and A^{12} are each hydrogen.

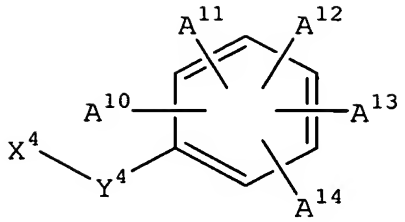
[0099] In another embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$; p is 0-4; q is 0; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are each hydrogen; A^{10}, A^{11} and A^{12} are each hydrogen; A^{13} is hydrogen, $-COOR^{43}$, C_1-C_4 alkyl, C_2-C_4 alkenyl or C_2-C_4 alkynyl; and A^{14} is $-COOR^{43}$.

[0100] In another embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is -S-; p and q are independently 1-4; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl or phenyl; A^{10}, A^{11} and A^{12} are each hydrogen; A^{13} is hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, phenyl, benzyl, phenoxy, benzyloxy or halo, wherein said alkyl, alkenyl, alkynyl, phenyl, benzyl, phenoxy and benzyloxy are independently unsubstituted or substituted with carobxy; and A^{14} is -COOH.

[0101] In another embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$, $-NR^{49}-$, -O-, -S- or $-SO_2-$; p and q are independently 0-4, provided that when q is 0 and W^2 is $-NR^{49}-$, -O-, -S- or $-SO_2-$, then Z^2 is $-CR^{41}R^{42}-$; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s); A^{10}, A^{11} and A^{12} are each hydrogen; A^{13} is hydrogen; and A^{14} is benzyl or carboxybenzyl.

FORMULA XIV

[0102] Another NAALADase inhibitor is a compound of formula XIV



XIV

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

X^4 is -(CO)NHOH or -N(OH)COH;

Y^4 is a bond or a divalent linking group having from 1 to 9 carbon atom(s) and from 0 to 5 heteroatom(s) independently selected from oxygen, sulfur and nitrogen;

A^{10}, A^{11}, A^{12}, A^{13} and A^{14} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, C_1-C_9 alkoxy, C_2-C_9 alkenyloxy, phenoxy, benzyloxy, hydroxy, halo, nitro, cyano, isocyano, -$COOR^{43}$, -COR^{43}, -$NR^{43}R^{44}$, -SR^{43}, -SOR^{43}, -SO_2R^{43}, -$SO_2(OR^{43})$, -$(CO)NR^{43}R^{44}$, -$(CO)NR^{43}(CH_2)_nCOOH$, -$NR^{43}(CO)R^{44}$ or -$(CH_2)_nCOOH$, or any adjacent two of A^{10}, A^{11}, A^{12} and A^{13} form with the benzene ring a fused ring that is saturated or unsaturated, aromatic or non-aromatic, and carbocyclic or heterocyclic, said heterocyclic ring containing 1 or 2 oxygen, nitrogen and/or sulfur heteroatom(s);

n is 1-3;

R^{43} and R^{44} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle or heterocycle; and

said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy, benzyloxy, and fused ring are independently unsubstituted or substituted with one or more substituent(s).

[0103] In one embodiment, Y^4 is a bond or -$(CR^{45}R^{46})_p$-W^2-$(CR^{47}R^{48})_q$-; W^2 is -$CR^{49}R^{50}$-, -NR^{49}-, -O-, -S- or -SO_2-; p and q are independently 0-4; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s); and A^{10}, A^{11} and A^{12} are each hydrogen.

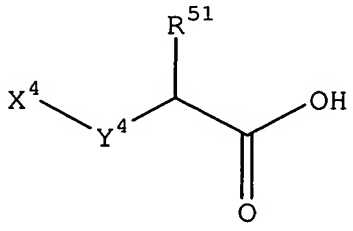
[0104] In another embodiment, Y^4 is a bond; A^{10}, A^{11} and A^{12} are each hydrogen; A^{13} is hydroxy, phenoxy, benzyloxy, $-COOR^{43}$ or $-(CO)NHR^{44}$; A^{14} is $-COOR^{43}$; R^{43} is hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl or C_2-C_4 alkynyl; R^{44} is benzyl; and said benzyl, phenoxy and benzyloxy are independently unsubstituted or substituted with $-COOR^{43}$.

[0105] In another embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is -O- or -S-; R^{45}, R^{46}, R^{47} and R^{48} are each hydrogen; A^{10}, A^{11} and A^{12} are each hydrogen; and A^{13} is hydrogen, -COOH, phenyl or benzyloxy, wherein said phenyl and benzyloxy are independently unsubstituted or substituted with $-COOR^{43}$; and A^{14} is $-COOR^{43}$.

[0106] In another embodiment, Y^4 is a bond or $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$, $-NR^{49}-$, -O-, -S- or $-SO_2-$; p and q are independently 0-4; R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s); A^{10}, A^{11} and A^{12} are each hydrogen; A^{13} is hydrogen, nitro or C_1-C_4 alkoxy; A^{14} is hydroxy, phenoxy, benzyloxy, benzoyl or C_1-C_4 alkoxy, wherein said phenoxy, benzyloxy, benzoyl and alkoxy are independently unsubstituted or substituted with one or more substituent(s).

FORMULA XV

[0107] Another NAALADase inhibitor is a compound of formula XV



XV

or an enantiomer or a pharmaceutically acceptable equivalent of said compound, wherein:

X^4 is $-(CO)NHOH$ or $-N(OH)COH$;

Y^4 is a bond or a divalent linking group having from 1 to 9 carbon atom(s) and from 0 to 5 heteroatom(s) independently selected from oxygen, sulfur and nitrogen; and

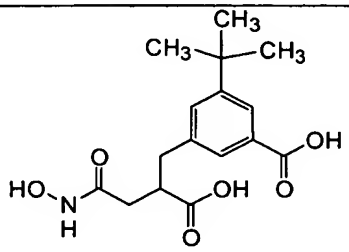
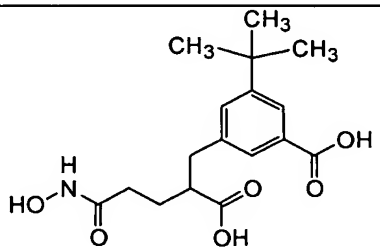
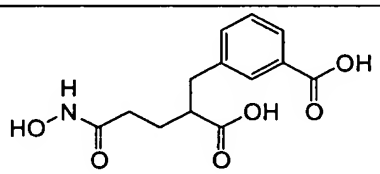
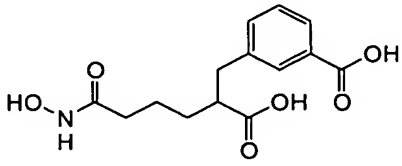
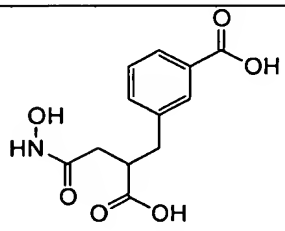
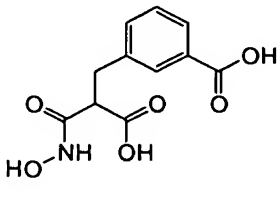
R^{51} is hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, C_1-C_9 alkoxy or C_2-C_9 alkenoxy, wherein said alkyl, alkenyl, alkynyl, alkoxy and alkenoxy are independently unsubstituted or substituted with one or more substituent(s); provided that when Y is methylene, amine or oxygen, then R^{51} is not carboxyethyl.

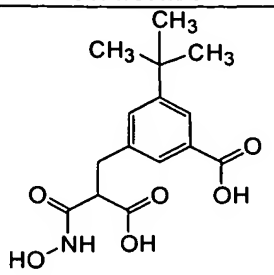
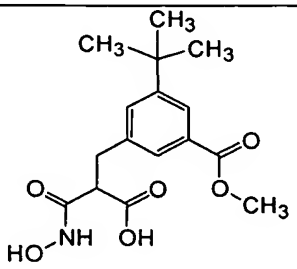
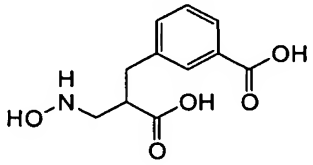
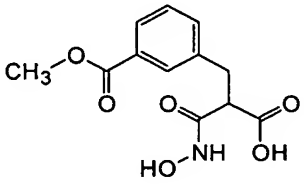
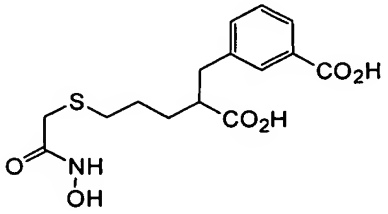
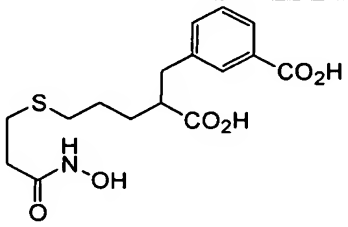
[0108] In one embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$, $-NR^{49}-$, -O-, -S- or $-SO_2-$; p and q are independently 0-4; and R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are independently hydrogen, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2-C_9 alkynyl, aryl, heteroaryl, carbocycle, heterocycle, halo, hydroxy, sulfhydryl, nitro, amino, cyano, isocyano, thiocyano, isothiocyano, formamido, thioformamido, sulfo, sulfino, C_1-C_9 alkoxy, C_2-C_9 alkenoxy, phenoxy or benzyloxy, wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, alkoxy, alkenyloxy, phenoxy and benzyloxy are independently unsubstituted or substituted with one or more substituent(s).

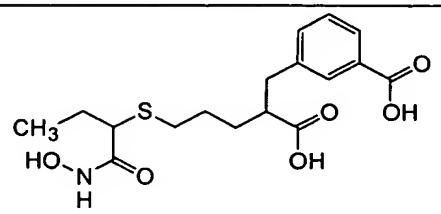
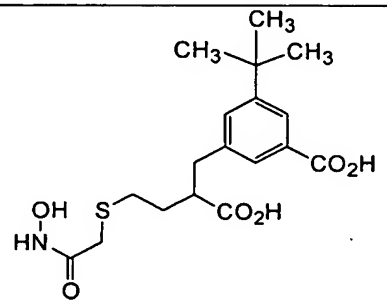
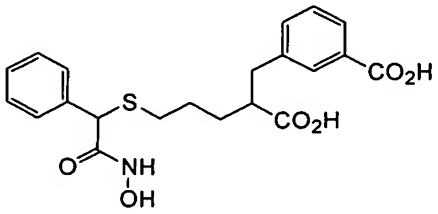
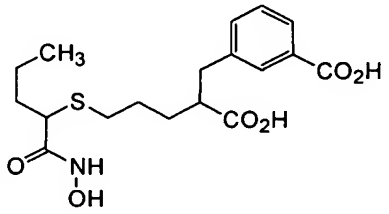
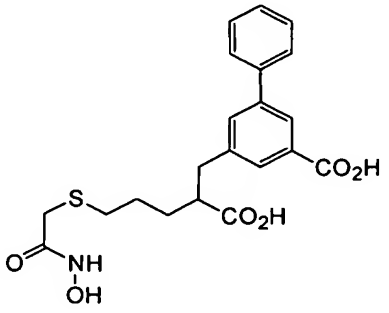
[0109] In another embodiment, Y^4 is $-(CR^{45}R^{46})_p-W^2-(CR^{47}R^{48})_q-$; W^2 is $-CR^{49}R^{50}-$ or -S-; p is 0-1; q is 0-3; and R^{45}, R^{46}, R^{47}, R^{48}, R^{49} and R^{50} are each hydrogen.

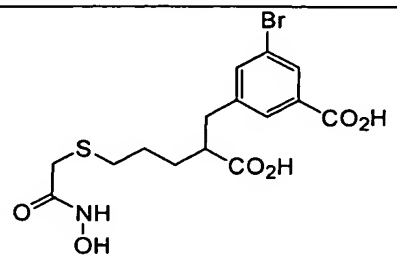
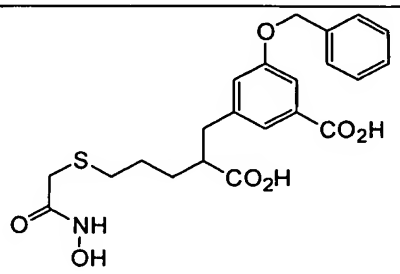
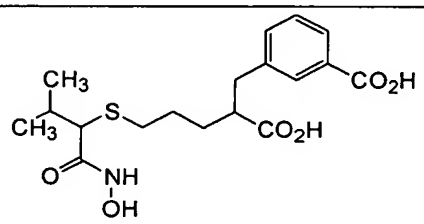
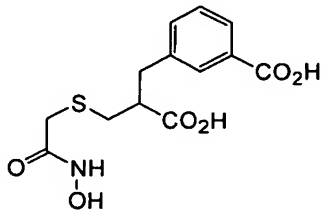
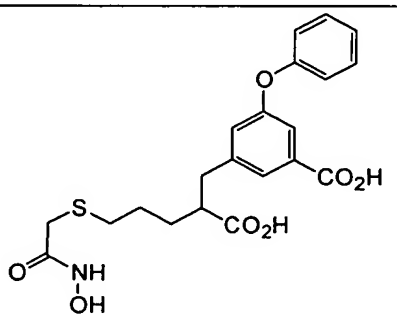
[0110] Examples of compounds of formulas XIII-XV are set forth below in TABLE I.

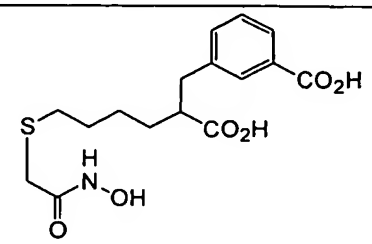
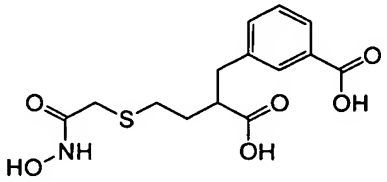
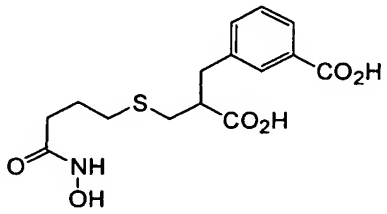
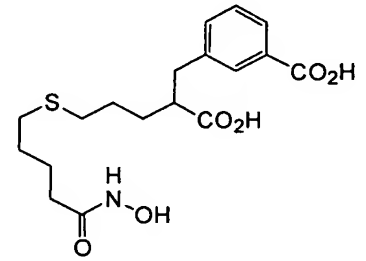
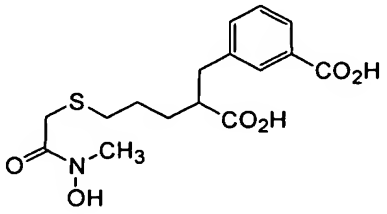
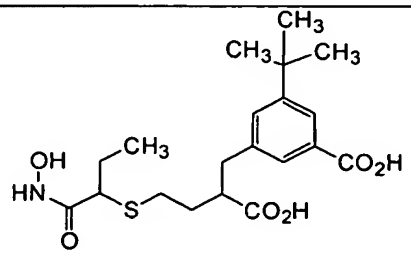
TABLE I

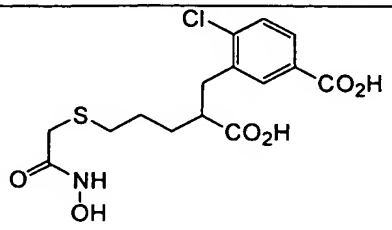
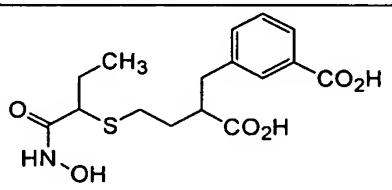
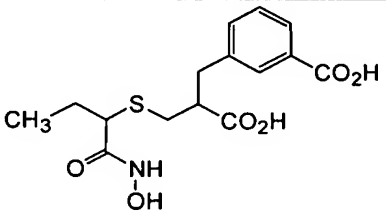
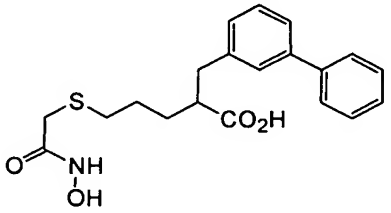
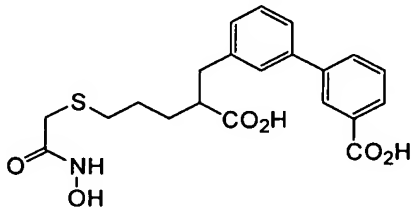
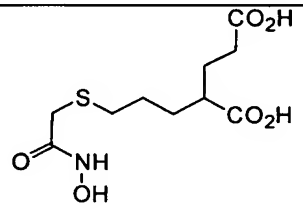
Structure	Name
	3-*tert*-Butyl-5-(2-carboxy-3-hydroxycarbamoyl-propyl)-benzoic acid
	3-*tert*-Butyl-5-(2-carboxy-4-hydroxycarbamoyl-butyl)-benzoic acid
	3-(2-Carboxy-4-hydroxycarbamoyl-butyl)-benzoic acid
	3-(2-Carboxy-5-hydroxycarbamoyl-pentyl)-benzoic acid
	3-(2-Carboxy-3-hydroxycarbamoyl-propyl)-benzoic acid
	3-(2-Carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid

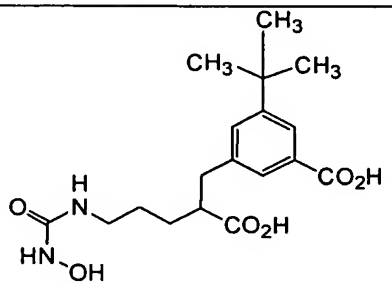
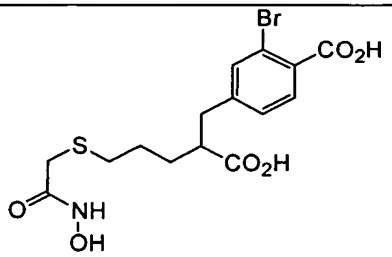
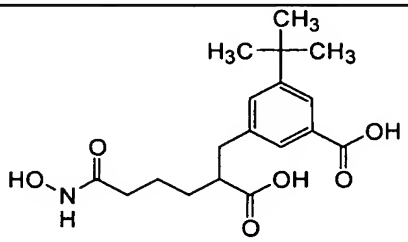
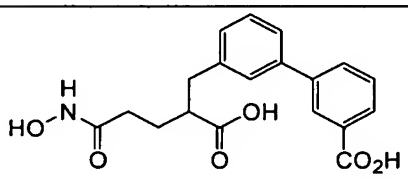
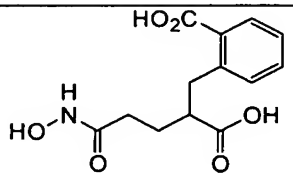
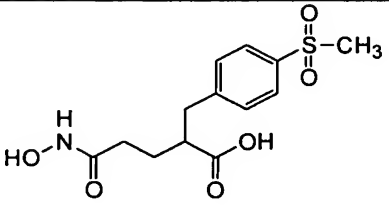
Structure	Name
	3-*tert*-Butyl-5-(2-carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid
	3-*tert*-Butyl-5-(2-carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid methyl ester
	3-(2-Carboxy-3-hydroxyamino-propyl)-benzoic acid
	3-(2-Carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid methyl ester
	3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid
	3-[2-Carboxy-5-(2-hydroxycarbamoyl-ethylsulfanyl)-pentyl]-benzoic acid

Structure	Name
	3-[2-Carboxy-5-(1-hydroxycarbamoyl-propylsulfanyl)-pentyl]-benzoic acid
	3-*tert*-Butyl-5-(2-carboxy-4-hydroxycarbamoylmethyl-sulfanylbutyl)-benzoic acid
	3-[2-Carboxy-5-(hydroxycarbamoyl-phenyl-methylsulfanyl)-pentyl]-benzoic acid
	3-[2-Carboxy-5-(1-hydroxycarbamoyl-butylsulfanyl)-pentyl]-benzoic acid
	5-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-biphenyl-3-carboxylic acid

Structure	Name
	3-Bromo-5-(2-carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid
	3-Benzyloxy-5-(2-carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid
	3-[2-Carboxy-5-(1-hydroxycarbamoyl-2-methyl-propylsulfanyl)-pentyl]-benzoic acid
	3-(2-Carboxy-3-hydroxycarbamoylmethyl-sulfanylpropyl)-benzoic acid
	3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-5-phenoxy-benzoic acid

Structure	Name
	3-(2-Carboxy-6-hydroxycarbamoylmethyl-sulfanylhexyl)-benzoic acid
	3-(2-Carboxy-4-hydroxycarbamoylmethyl-sulfanylbutyl)-benzoic acid
	3-[2-Carboxy-3-(3-hydroxycarbamoyl-propylsulfanyl)-propyl]-benzoic acid
	3-[2-Carboxy-5-(4-hydroxycarbamoyl-butylsulfanyl)-pentyl]-benzoic acid
	3-{2-Carboxy-5-[(hydroxy-methyl-carbamoyl)-methylsulfanyl]-pentyl}-benzoic acid
	3-*tert*-Butyl-5-[2-carboxy-4-(1-hydroxycarbamoyl-propylsulfanyl)-butyl]-benzoic acid

Structure	Name
	3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-4-chloro-benzoic acid
	3-[2-Carboxy-4-(1-hydroxycarbamoyl-propylsulfanyl)-butyl]-benzoic acid
	3-[2-Carboxy-3-(1-hydroxycarbamoyl-propylsulfanyl)-propyl]-benzoic acid
	2-Biphenyl-3-ylmethyl-5-hydroxycarbamoylmethylsulfanyl-pentanoic acid
	3'-(2-Carboxy-5-hydroxycarbamoylmethylsulfanyl-pentyl)-biphenyl-3-carboxylic acid
	2-(3-Hydroxycarbamoyl-methylsulfanylpropyl)-pentanedioic acid

Structure	Name
	3-(2-Carboxy-5-{[(hydroxy-amino)carbonyl]amino}-pentyl)-5-*tert*-butylbenzoic acid
	2-Bromo-4-(2-carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid
	
	
	
	

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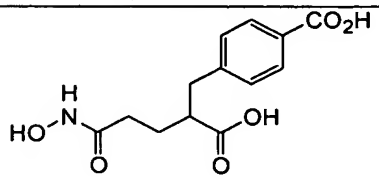
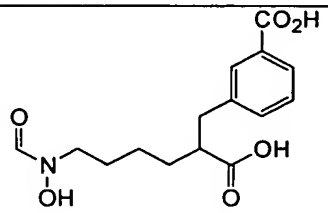
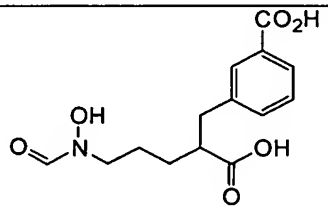
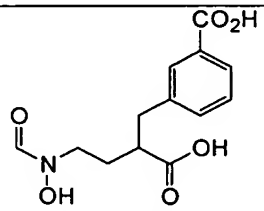
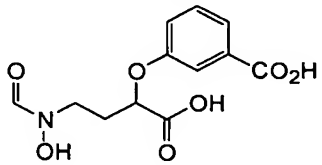
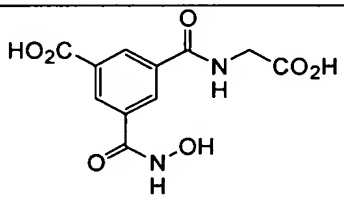
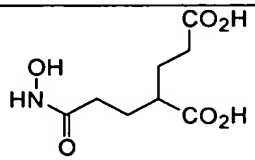
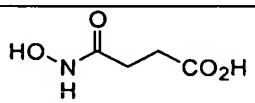
	4-[2-carboxy-5-(hydroxyamino)-5-oxopentyl]benzoic acid
	
	
	
	
	
	
	

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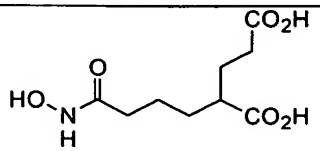
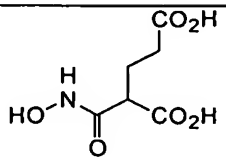
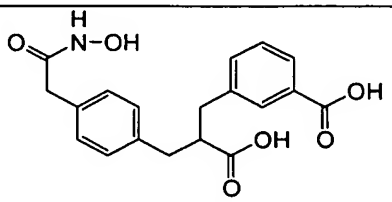
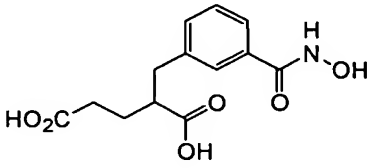
	
	
	
	

OTHER NAALADASE INHIBITORS

[0111] Other NAALADase inhibitors are described in International Publication No. WO 01/14390 and U.S. Patent No. 6,348,464, the entire contents of which publication and patent are herein incorporated by reference as though set forth herein in full.

[0112] Possible substituents of the compounds of formulas I-XV include, without limitation, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, C_2-C_6 alkenyloxy, phenoxy, benzyloxy, hydroxy, carboxy, hydroperoxy, carbamido, carbamoyl, carbamyl, carbonyl, carbozoyl, amino, hydroxyamino, formamido, formyl, guanyl, cyano, cyanoamino, isocyano, isocyanato, diazo, azido, hydrazino, triazano, nitrilo, nitro, nitroso, isonitroso, nitrosamino, imino, nitrosimino, oxo, C_1-C_6 alkylthio, sulfamino, sulfamoyl, sulfeno, sulfhydryl, sulfinyl, sulfo, sulfonyl, thiocarboxy, thiocyano, isothiocyano, thioformamido, halo, haloalkyl, chlorosyl, chloryl, perchloryl, trifluoromethyl, iodosyl, iodyl, phosphino, phosphinyl, phospho, phosphono, arsino, selanyl, disilanyl, siloxy, silyl, silylene and carbocyclic and heterocyclic moieties.

[0113] Carbocyclic moieties include alicyclic and aromatic structures. Examples of carbocyclic and heterocyclic moieties include, without limitation, phenyl, benzyl, naphthyl, indenyl, azulenyl, fluorenyl, anthracenyl, indolyl, isoindolyl, indolinyl, benzofuranyl, benzothiophenyl, indazolyl, benzimidazolyl, benzthiazolyl, tetrahydrofuranyl, tetrahydropyranyl, pyridyl, pyrrolyl, pyrrolidinyl, pyridinyl, pyrimidinyl, purinyl, quinolinyl, isoquinolinyl, tetrahydroquinolinyl, quinolizinyl, furyl, thiophenyl, imidazolyl, oxazolyl, benzoxazolyl, thiazolyl, isoxazolyl, isotriazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, trithianyl, indolizinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, thienyl, tetrahydroisoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, naphthyridinyl, pteridinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, and phenoxazinyl.

[0114] All variables of formulas I-XV are independently selected at each occurrence. For example, formula II may have two different $CR^{10}R^{11}$ moieties when X is a moiety of formula III and n is 2, with the first $CR^{10}R^{11}$ moiety being CH_2, and the second $CR^{10}R^{11}$ moiety being $CH(CH_3)$.

[0115] The compounds of formulas I-XV may possess one or more asymmetric carbon center(s) and, thus, may be capable of existing in the form of optical isomers as well as in the form of racemic or non-racemic mixtures of optical isomers. The optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes well known in the art, for example by formation of diastereoisomeric salts by treatment with an optically active acid or base, and then separation of the mixture of diastereoisomers by crystallization followed by liberation of the optically active bases from these salts. Examples of optically active acids are tartaric, diacetyltartaric, dibenzoyltartaric, ditoluoyltartaric and camphorsulfonic acid. A different process for separation of optical isomers involves the use of a chiral chromatography column optimally chosen to maximize the separation of the enantiomers. Still another available method involves synthesis of covalent diastereoisomeric molecules, for example, esters, amides, acetals, ketals, and the like, by reacting compounds used in the inventive methods and pharmaceutical compositions with an optically active acid in an activated form, an optically active diol or an optically active isocyanate. The synthesized diastereoisomers can be separated by conventional means such as chromatography, distillation, crystallization or sublimation, and then

hydrolyzed to deliver the enantiomerically pure compound. In some cases hydrolysis to the parent optically active drug is not necessary prior to dosing the patient since the compound can behave as a prodrug. The optically active compounds can likewise be obtained by utilizing optically active starting materials.

[0116] It is understood that the compounds of formulas I-XV encompass optical isomers as well as racemic and non-racemic mixtures.

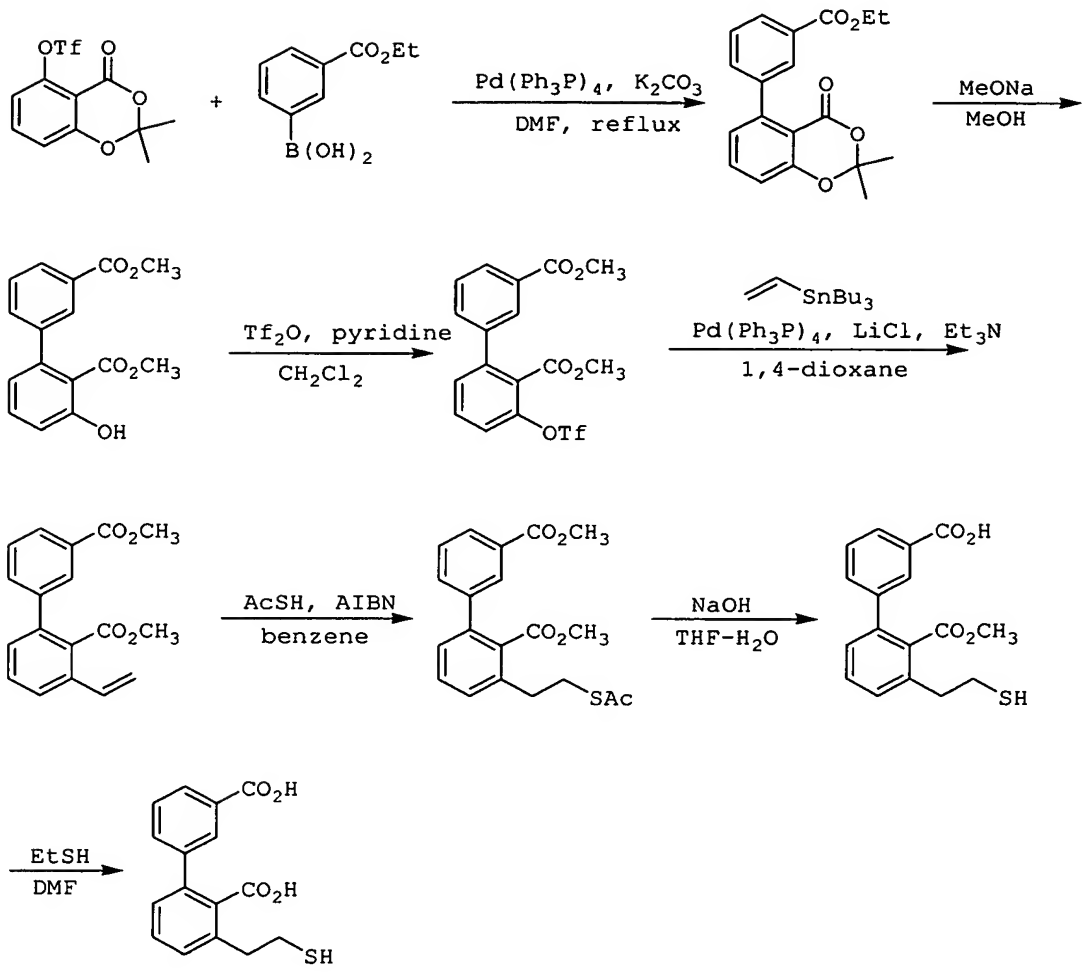
SYNTHESIS OF NAALADASE INHIBITORS

[0117] Some of the NAALADase inhibitors used in the inventive methods and pharmaceutical compositions can be readily prepared by standard techniques of organic chemistry, utilizing the general synthetic pathways and examples depicted in U.S. Patents Nos. 5,672,592, 5,795,877, 5,863,536, 5,880,112, 5,902,817, 5,962,521, 5,968,915, 6,025,344, 6,025,345, 6,028,216, 6,046,180, 6,054,444, 6,071,965, 6,121,252, 6,265,609, 6,348,464, 6,452,044, 6,458,775, 6,586,623, and International Publications Nos. WO 01/14390, WO 02/096866, WO 03/057670 and WO 02/092553, the entire contents of which patents and publications are herein incorporated by reference, as though set forth herein in full.

[0118] Other NAALADase inhibitors may be available from commercial suppliers or can be readily prepared by an ordinarily skilled artisan using standard techniques such as those disclosed in U.S. Patent No. 5,859,046, the entire contents of which reference are herein incorporated by reference as though set forth herein in full.

[0119] Yet other NAALADase inhibitors can be readily prepared by standard techniques of organic chemistry, utilizing the general synthetic pathways depicted below in SCHEMES I-XIV.

SCHEME I


OTf
O
O
O
+
CO2Et
B(OH)2
Pd(Ph3P)4, K2CO3
DMF, reflux
CO2Et
O
O
O
MeONa
MeOH
CO2CH3
CO2CH3
OH
Tf2O, pyridine
CH2Cl2
CO2CH3
CO2CH3
OTf
SnBu3
Pd(Ph3P)4, LiCl, Et3N
1,4-dioxane
CO2CH3
CO2CH3
AcSH, AIBN
benzene
CO2CH3
CO2CH3
SAc
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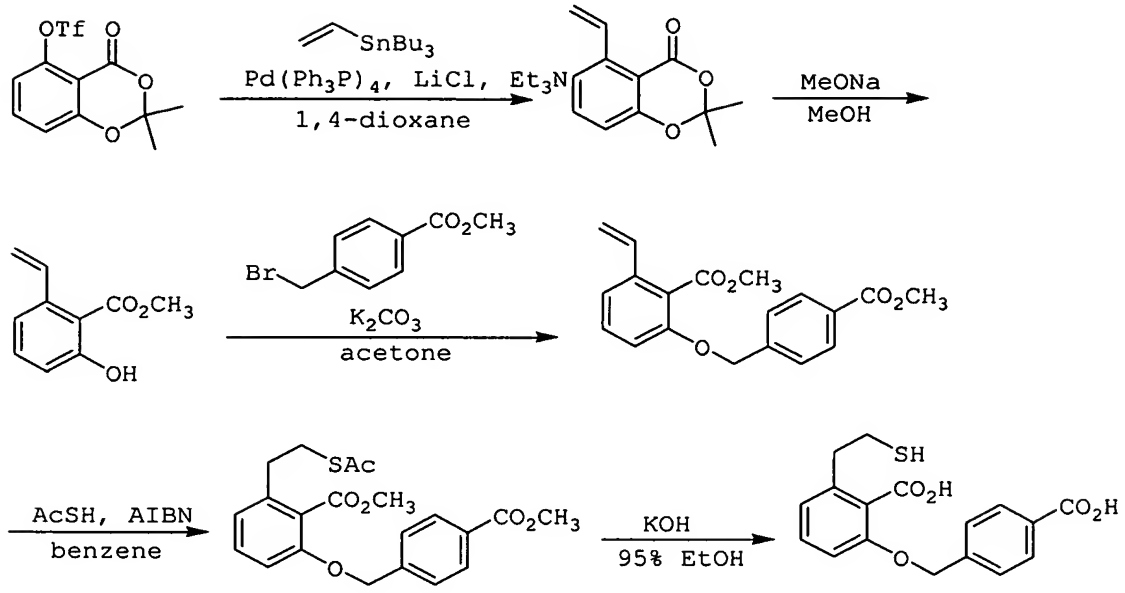
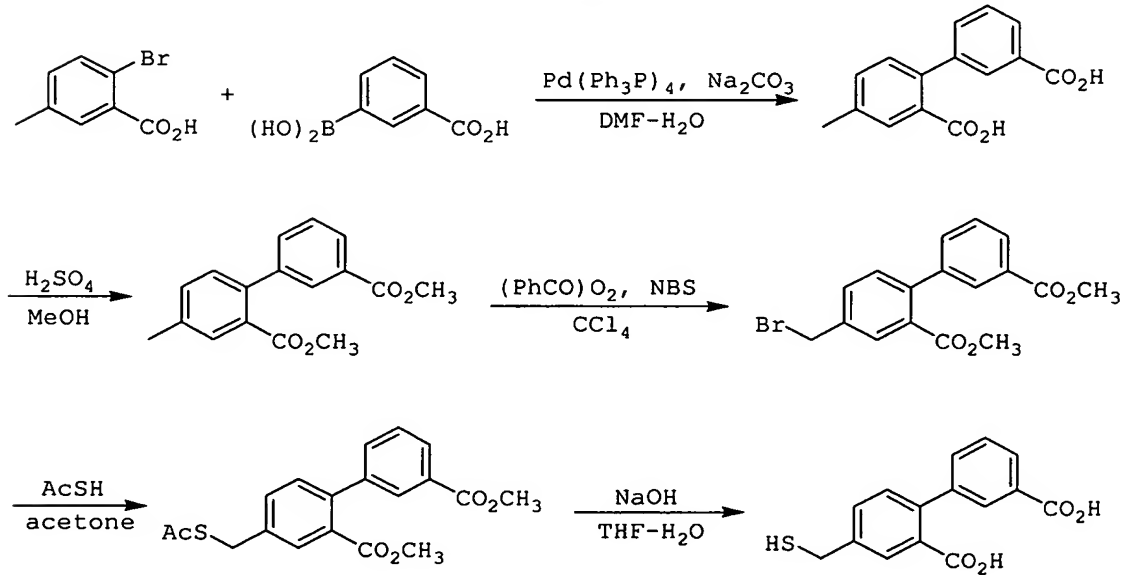
SH
EtSH
DMF
CO2H
CO2H
SH

SCHEME II



SCHEME III



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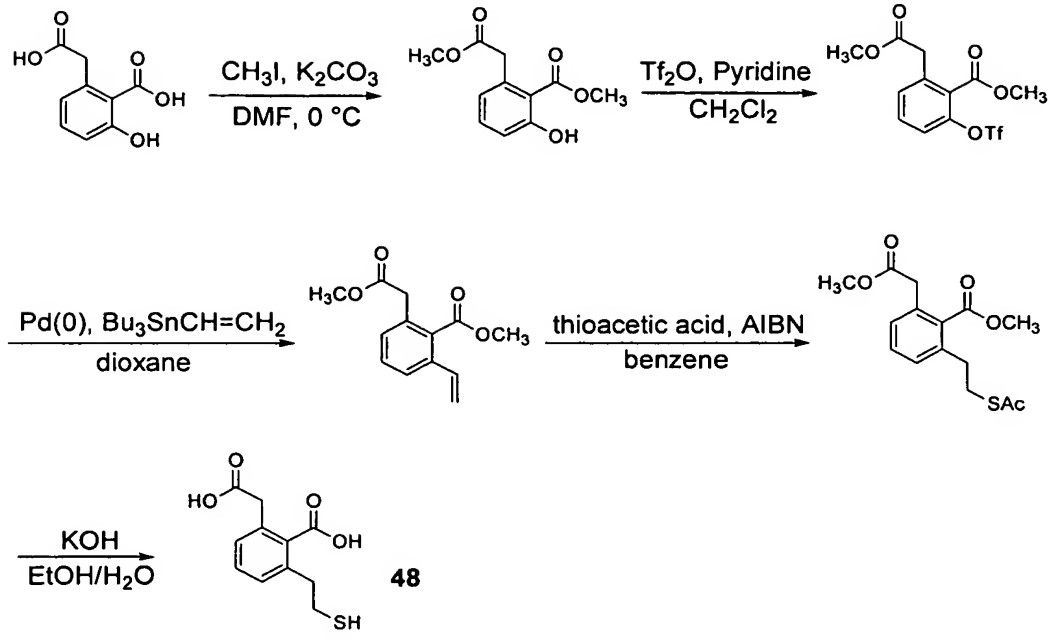
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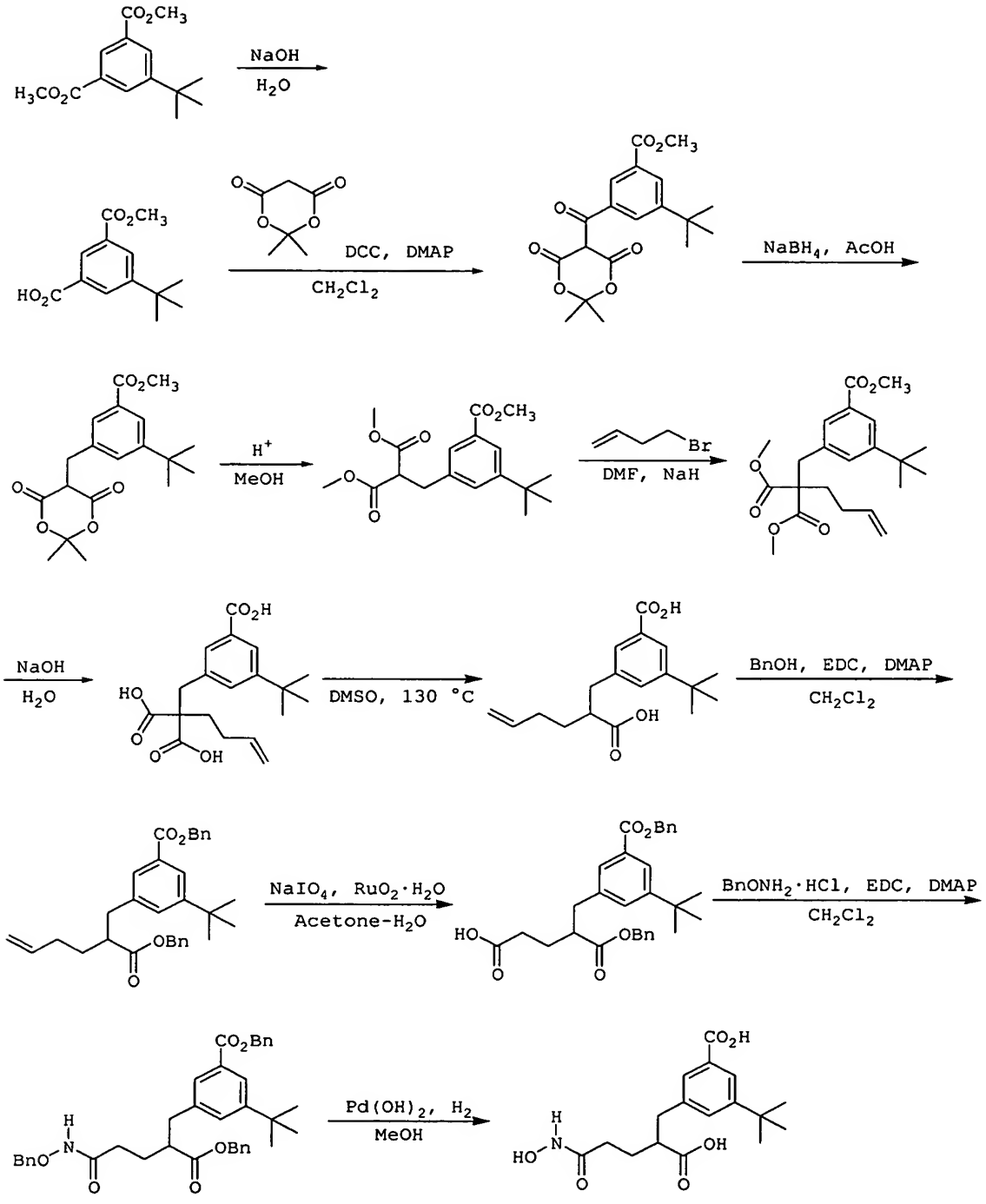
O
OH
OH
CH3I, K2CO3
DMF, 0 °C
H3CO
OCH3
Tf2O, Pyridine
CH2Cl2
OTf
Pd(0), Bu3SnCH=CH2
dioxane
thioacetic acid, AIBN
benzene
SAc
KOH
EtOH/H2O
SH
48

SCHEME V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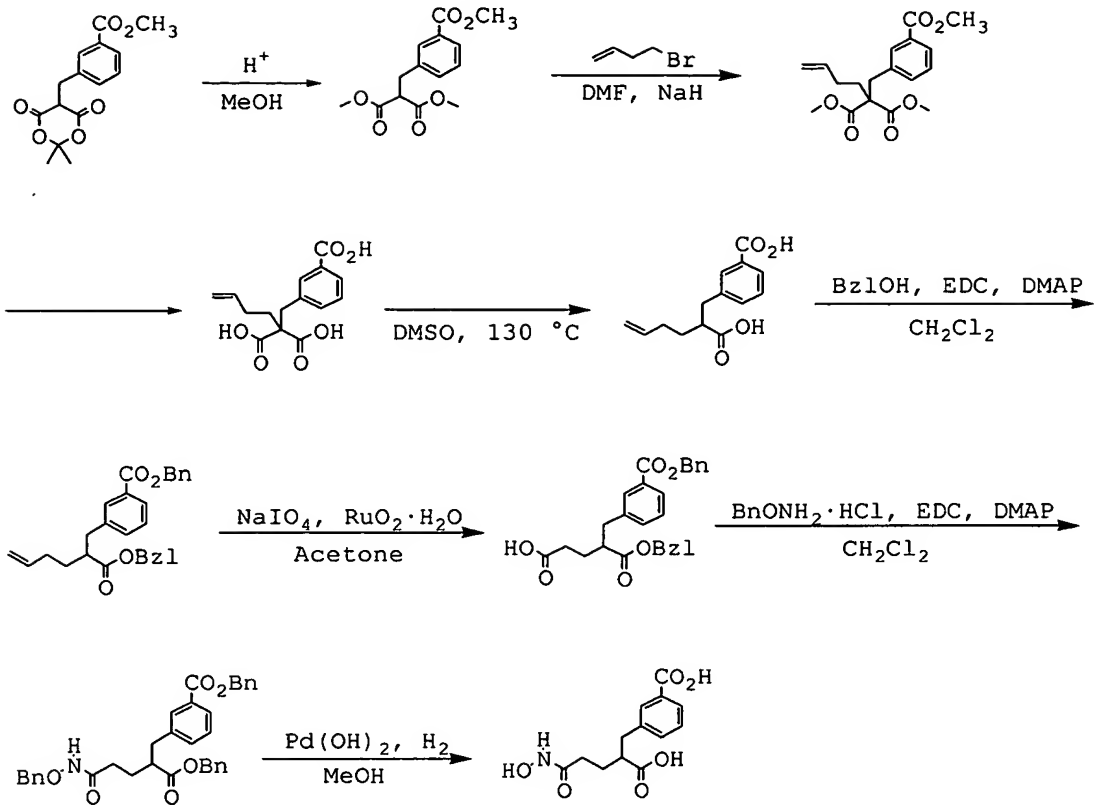
H3CO2C
NaOH
H2O
HO2C
DCC, DMAP
CH2Cl2
NaBH4, AcOH
H+
MeOH
Br
DMF, NaH
CO2H
HO
OH
DMSO, 130 °C
BnOH, EDC, DMAP
CO2Bn
OBn
NaIO4, RuO2·H2O
Acetone-H2O
BnONH2·HCl, EDC, DMAP
BnO
Pd(OH)2, H2
MeOH

SCHEME VI



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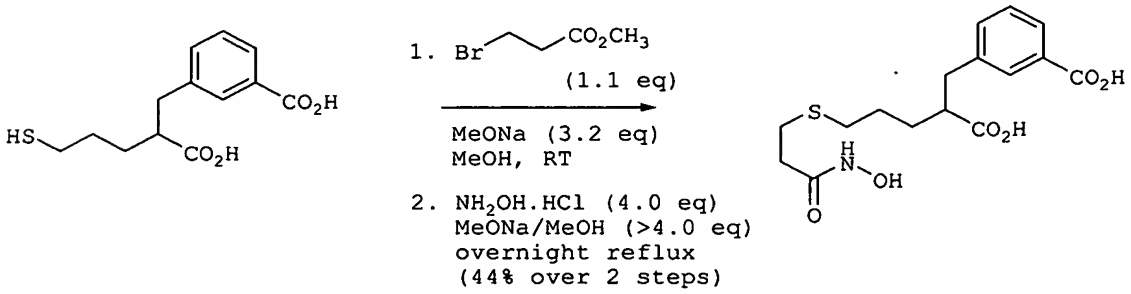

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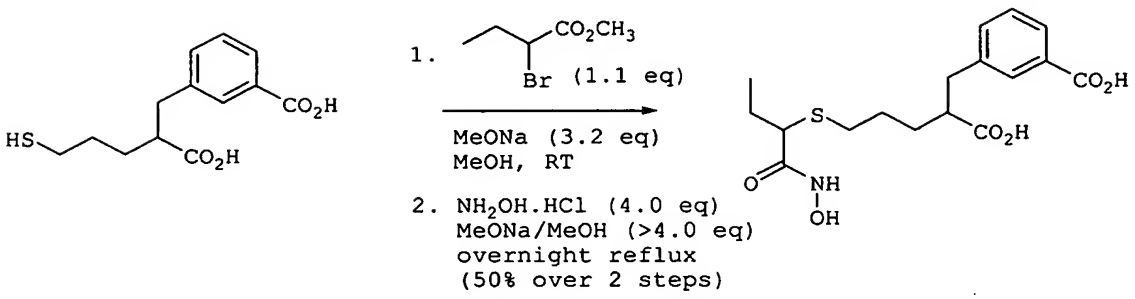
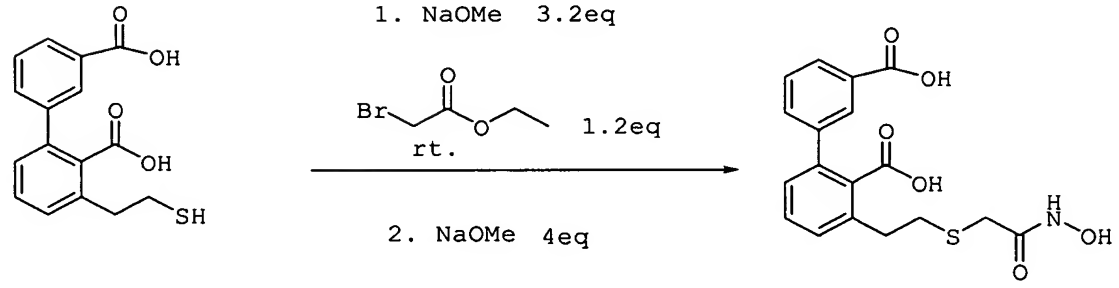
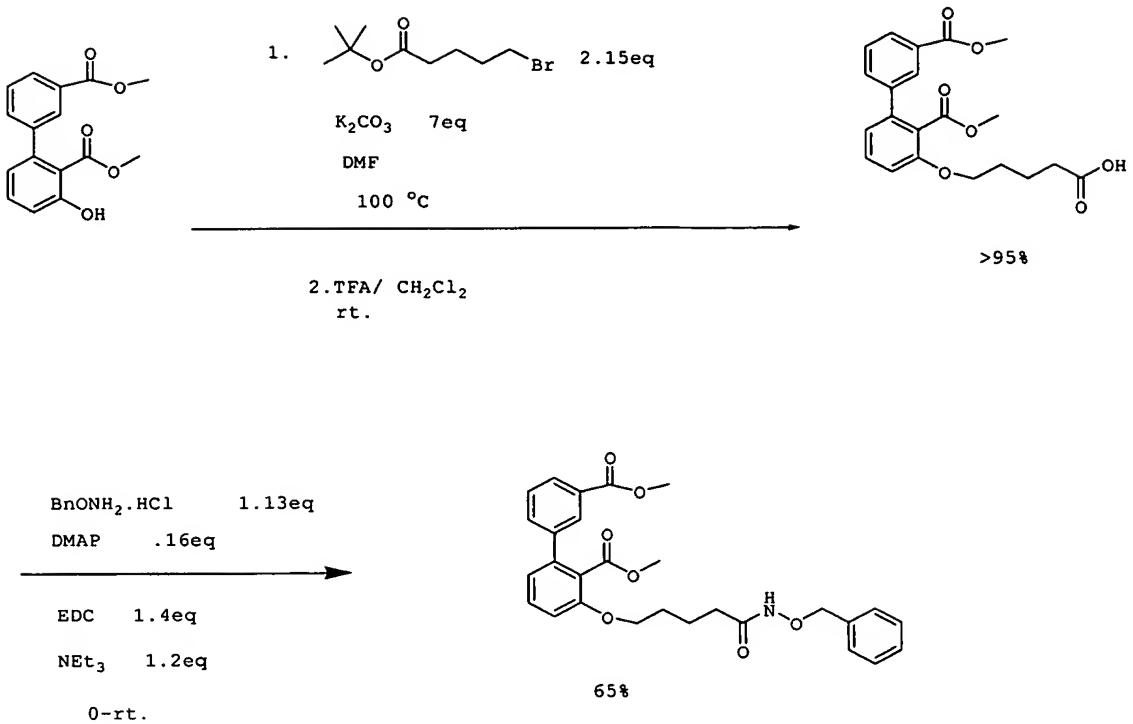


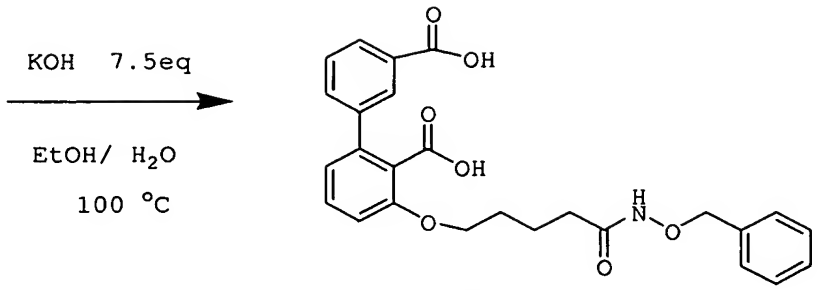
SCHEME IX



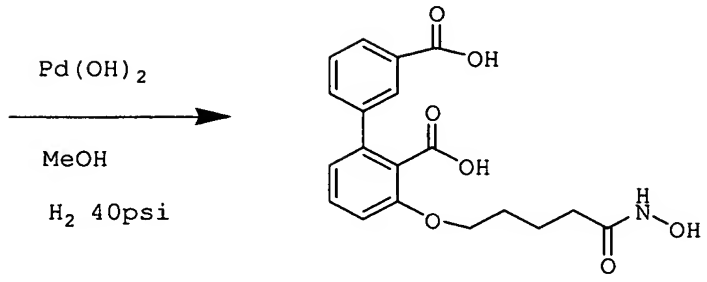
15.7%

SCHEME X


1.
2.15eq
K_2CO_3 7eq
DMF
100 °C
2.TFA/ CH_2Cl_2
rt.
>95%
$BnONH_2.HCl$ 1.13eq
DMAP .16eq
EDC 1.4eq
NEt_3 1.2eq
0-rt.
65%



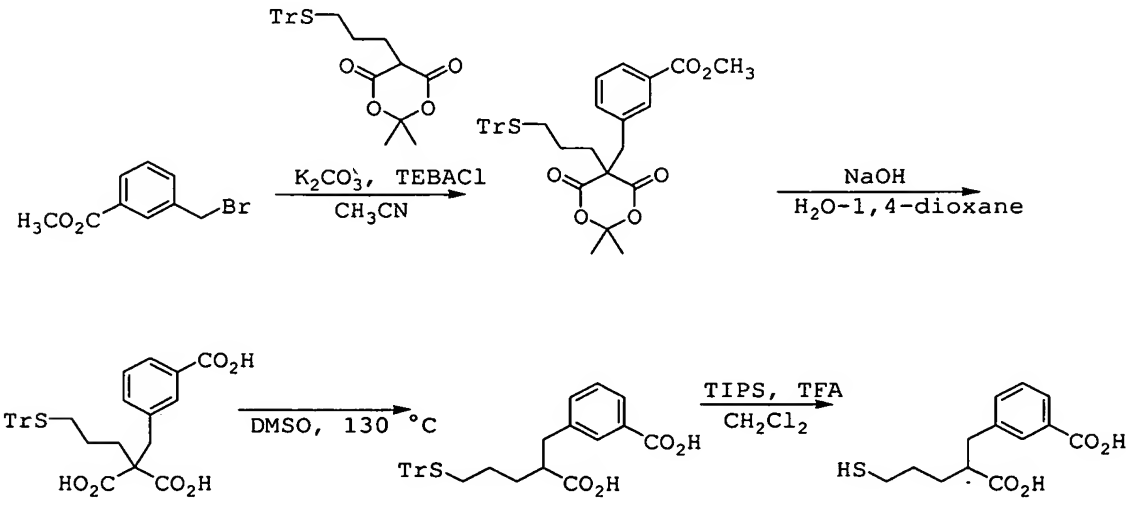
62%



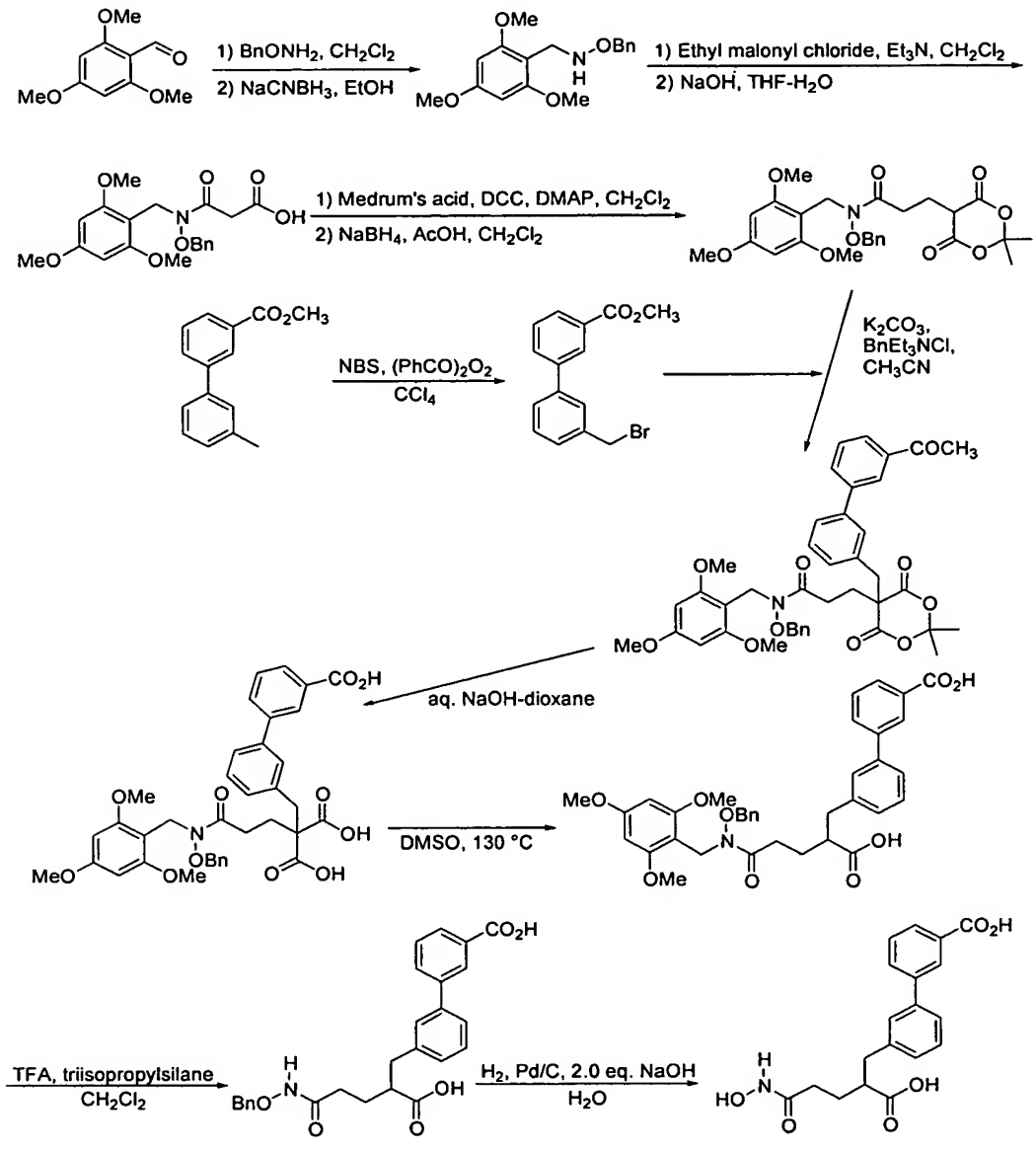
83%

[0120] Precursor compounds may be commercially available, prepared by methods known to a person of skill in the art, or prepared by SCHEMES XI to XIV.

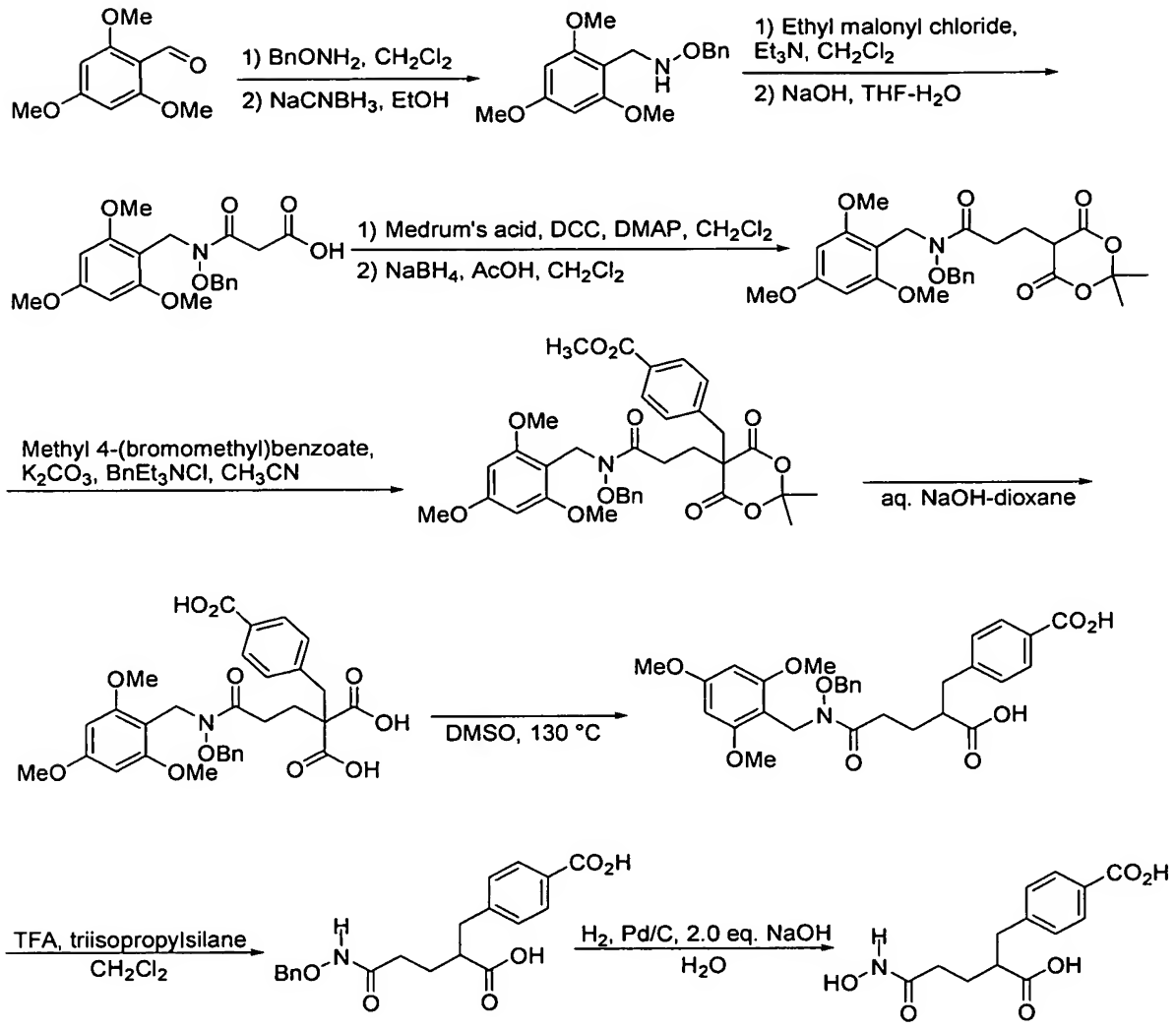
SCHEME XI



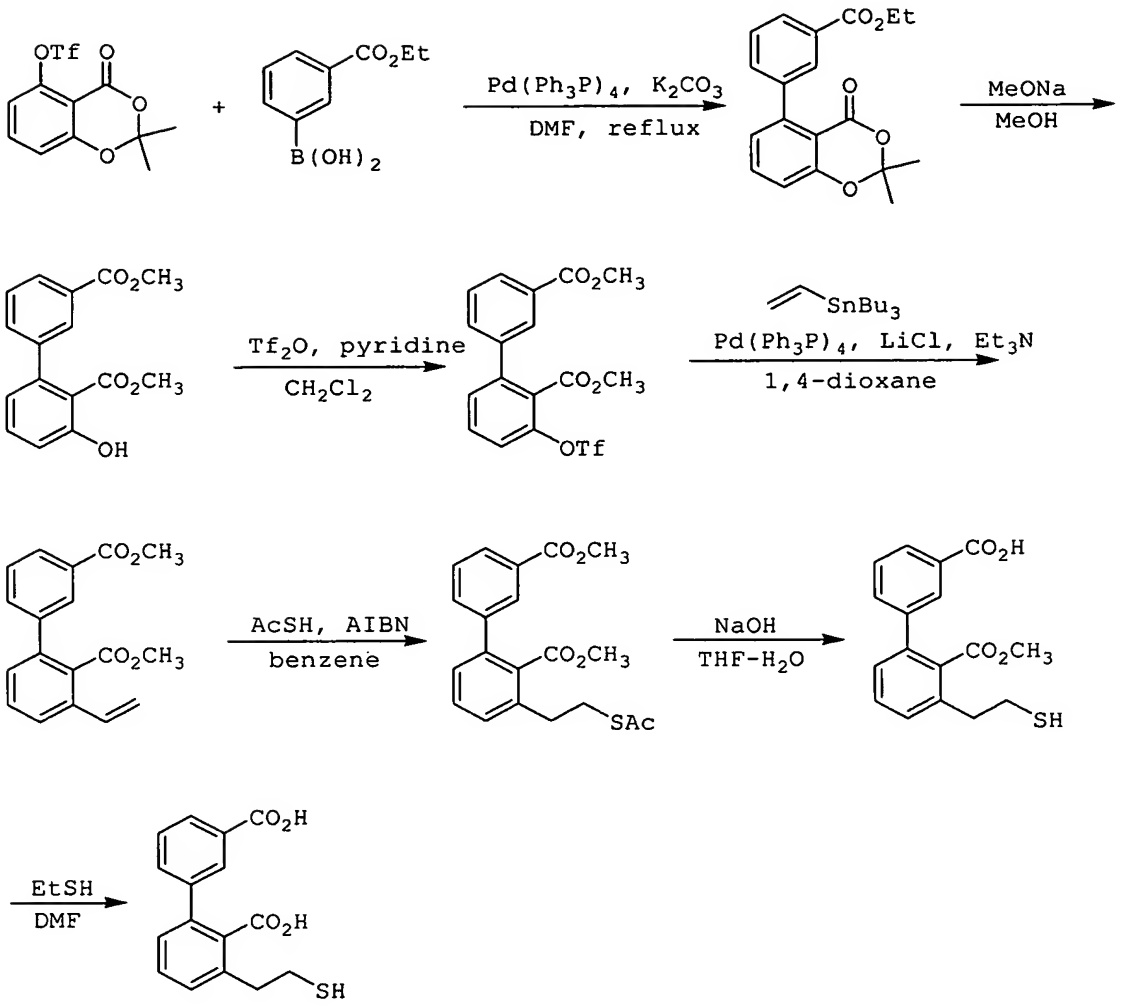
SCHEME XII


1) BnONH2, CH2Cl2
2) NaCNBH3, EtOH
1) Ethyl malonyl chloride, Et3N, CH2Cl2
2) NaOH, THF-H2O
1) Medrum's acid, DCC, DMAP, CH2Cl2
2) NaBH4, AcOH, CH2Cl2
NBS, (PhCO)2O2
CCl4
K2CO3,
BnEt3NCl,
CH3CN
aq. NaOH-dioxane
DMSO, 130 °C
TFA, triisopropylsilane
CH2Cl2
H2, Pd/C, 2.0 eq. NaOH
H2O

SCHEME XIII


1) BnONH2, CH2Cl2
2) NaCNBH3, EtOH
1) Ethyl malonyl chloride, Et3N, CH2Cl2
2) NaOH, THF-H2O
1) Medrum's acid, DCC, DMAP, CH2Cl2
2) NaBH4, AcOH, CH2Cl2
Methyl 4-(bromomethyl)benzoate, K2CO3, BnEt3NCl, CH3CN
aq. NaOH-dioxane
DMSO, 130 °C
TFA, triisopropylsilane
CH2Cl2
H2, Pd/C, 2.0 eq. NaOH
H2O

SCHEME XIV


OTf
O
O
O
+
CO2Et
B(OH)2
Pd(Ph3P)4, K2CO3
DMF, reflux
CO2Et
O
O
O
MeONa
MeOH
CO2CH3
CO2CH3
OH
Tf2O, pyridine
CH2Cl2
CO2CH3
CO2CH3
OTf
SnBu3
Pd(Ph3P)4, LiCl, Et3N
1,4-dioxane
CO2CH3
CO2CH3
AcSH, AIBN
benzene
CO2CH3
CO2CH3
SAc
NaOH
THF-H2O
CO2H
CO2CH3
SH
EtSH
DMF
CO2H
CO2H
SH

ROUTE OF ADMINISTRATION

[0121] In the inventive methods, the compounds will generally be administered to a patient in the form of a pharmaceutical formulation. Such formulation may include, in addition to the active agent, a physiologically acceptable carrier and/or diluent. The compounds may be administered locally or systemically by any means known to an ordinarily skilled artisan. For example, the compounds may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intraarterial, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal, intracranial or intraosseous injection and infusion techniques. Topical administration includes, without limitation, administration via eyedrops. The exact administration protocol will vary depending upon various factors including the age, body weight, general health, sex and diet of the patient; the determination of specific administration procedures would be routine to an ordinarily skilled artisan. The compounds and compositions used in the inventive methods may be capable of crossing the blood-brain barrier.

DOSAGE

[0122] In the inventive methods, the compounds and compositions may be administered by a single dose, multiple discrete doses or continuous infusion. Pump means, particularly subcutaneous pump means, are useful for continuous infusion.

[0123] Dose levels on the order of about 0.001 to about 10,000 mg/kg of the active ingredient compound are useful in the inventive methods. In one embodiment, the dose level is about 0.1 mg/kg/d to about 1,000 mg/kg/d. In another embodiment, the dose level is about 1 mg/kg/d to about 100 mg/kg/d. The specific dose level for any particular patient will vary depending upon a variety of factors, including the activity and the possible toxicity of the specific compound employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the rate of excretion; drug combination; the severity of the particular disease being treated; and the form of administration. Typically, *in vitro* dosage-effect results provide useful guidance on the proper doses for patient administration.

Studies in animal models are also helpful. The considerations for determining the proper dose levels are well known in the art.

ADMINISTRATION REGIMEN

[0124] For the inventive methods, any administration regimen well known to an ordinarily skilled artisan for regulating the timing and sequence of drug delivery can be used and repeated as necessary to effect treatment. Such regimen may include pretreatment and/or co-administration with additional therapeutic agents.

CO-ADMINISTRATION WITH OTHER TREATMENTS

[0125] In the inventive methods, the NAALADase inhibitors and pharmaceutical compositions may be used alone or in combination with one or more additional agent(s) for simultaneous, separate or sequential use.

[0126] The additional agent(s) may be any therapeutic agent(s) known to an ordinarily skilled artisan, including, without limitation, (an)other compound(s) of formulas I-XV.

[0127] The NAALADase inhibitors and pharmaceutical compositions may be co-administered with one or more therapeutic agent(s) either (i) together in a single formulation, or (ii) separately in individual formulations designed for optimal release rates of their respective active agent. Each formulation may contain from about 0.01% to about 99.99% by weight of a NAALADase inhibitor, as well as one or more pharmaceutically acceptable carrier(s), such as wetting, emulsifying and/or pH buffering agent(s).

[0128] In addition, the NAALADase inhibitors and pharmaceutical compositions may be administered prior to, during or following surgery or physical therapy.

EXAMPLES

[0129] The following examples are illustrative of the present invention and are not intended to be limitations thereon. Unless otherwise indicated, all percentages are based upon 100% by weight of the final composition.

EXAMPLE 1

Preparation 3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic Acid

(Scheme I)

3-(2,2-Dimethyl-4-oxo-4*H*-1,3-benzodioxin-5-yl)-benzoic acid, ethyl ester

[0130] To a solution of 2,2-dimethyl-5-trifluoro-methanesulfonyloxy-4*H*-1,3-benzodioxin-4-one (2.0 g, 5.8 mmol), 3-ethoxycarbonylphenylboronic acid (1.34 g, 6.9 mmol) and anhydrous K_2CO_3 powder (2.61 g, 18.9 mmol) in DMF (30 mL) was added tetrakis(triphenylphosphine) palladium(0.202 g, 0.175 mmol). The mixture was heated at reflux for 2 hours. The reaction mixture was allowed to cool to room temperature and 1 N HCl (25 mL) was added. The mixture was extracted with EtOAc (3 X 25 mL). The combined extracts were washed with water and brine, then dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by flash chromatography (1:15 EtOAc/hexanes) to afford 3-(2,2-dimethyl-4-oxo-4H-1,3-benzodioxin-5-yl)-benzoic acid, ethyl ester (1.2 g, 63%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.1 Hz, 3H), 1.80 (s, 6H), 4.39 (q, J = 7.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 2H), 7.47-7.57 (m, 3H), 8.00 (t, J = 1.5 Hz, 1H), 8.07 (dt, J = 7.5, 1.5 Hz, 1H).

3-Hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0131] To a solution of 3-(2,2-dimethyl-4-oxo-4*H*-1,3-benzodioxin-5-yl)benzoic acid, ethyl ester (1.4 g, 4.3 mmol) in methanol (10 mL) was added sodium methoxide (0.5 M in methanol, 25 mL) at 0 °C. The solution was stirred at room temperature for 15 minutes. The reaction was quenched by addition of 1 N HCl (30 mL) and extracted with EtOAc (3 X 30 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated to afford 3-hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.2 g, 95%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 3H), 3.93 (s, 3H), 6.79 (dd, J = 7.5, 0.9 Hz, 1H), 7.04 (dd, J = 7.5, 0.9 Hz, 1H), 7.43 (m, 3H), 7.93 (m, 1H), 8.02 (dm, J = 7.0 Hz, 1H), 10.8 (s, 1H).

3-Trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0132] To a solution of 3-hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.1 g, 3.8 mmol) in dichloromethane (15 mL) were added pyridine (1.00 mL, 12.3 mmol) and trifluromethanesulfonic anhydride (0.90 mL, 5.4 mmol) at 0 °C. The solution was stirred at 0 °C for 2 hours. Aqueous 1 N HCl (20 mL) was added, and the mixture was extracted with dichloromethane (3 X 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to give 3-trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.4 g, 87%) as a yellow solid: ^{1}H NMR ($CDCl_3$) □3.72 (s, 3H), 3.94 (s, 3H), 7.38-7.62 (m, 5H), 8.08 (m, 2H).

3-Ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0133] A mixture of 3-trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.3 g, 3.1 mmol), tetrakis(triphenylphosphine)palladium (0.36 g, 0.31 mmol), LiCl (0.94 g, 22.2 mmol), triethylamine (0.6 mL, 4.3 mmol) and tri-n-butyl(vinyl)tin (1.0 mL, 3.4 mmol) in 1,4-dioxane (30 mL) was heated at reflux under N_2 for 4 hours. After cooling to room temperature, the mixture was filtered through a plug of silica gel and the filtrate was concentrated. Purification by flash chromatography (1:10 EtOAc/hexanes) provided 3-ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.91 g, 99%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 3H), 3.92 (s, 3H), 5.40 (d, J = 11.1 Hz, 1H), 5.79 (d, J = 17.5 Hz, 1H), 6.87 (dd, J = 17.4, 11.0 Hz, 1H), 7.31 (d, J = 7.5 Hz, 1H), 7.44-7.49 (m, 2H), 7.56 (dm, J = 7.5 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 8.03 (dm, J = 7.5 Hz, 1H), 8.08 (t, 1, J = 1.5 Hz, 1H).

3-[2-(Acetylthio)ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0134] To a solution of 3-ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.85 g, 2.9 mmol) in benzene (10 mL) was added thioacetic acid (2.1 mL, 29.4 mmol) followed by AIBN (0.053 g, 0.32 mmol). The solution was deoxygenated for 30 minutes by bubbling nitrogen through the solution and then heated at reflux for 4 hours. Saturated aqueous $NaHCO_3$ (20 mL) was added to the solution and the mixture was extracted with EtOAc (2 X 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (1:12 EtOAc/hexanes) to give 3-[2-(acetylthio)ethyl]-[1,1'-biphenyl]-2,3'-

dicarboxylic acid, dimethyl ester (0.51 g, 48%) as an off white solid: ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H), 2.93 (m, 2H), 3.14 (m, 2H), 3.62 (s, 3H), 3.93 (s, 3H), 7.29 (dd, J = 7.6, 0.9 Hz, 1H), 7.35 (dd, J = 7.5, 0.8 Hz, 1H), 7.44 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.55 (dt, J = 8.0, 1.5 Hz, 1H), 8.03 (dt, J = 7.9, 1.5 Hz, 1H), 8.07 (t, J = 1.5 Hz, 1H).

3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester

[0135] To a deoxygenated solution of 3-[2-(acetylthio) ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.50 g, 1.34 mmol) in THF (3.5 mL) was added a deoxygenated solution of NaOH (0.38 g, 9.4 mmol) in water (3.5 mL). The mixture was stirred overnight, and 1 N HCl (20 mL) was added. The mixture was extracted with EtOAc (3 X 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to afford 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester (0.35 g, 83%) as an off white solid: ^{1}H NMR ($CDCl_3$) δ 1.46 (t, J = 8.0 Hz, 1H), 2.83 (m, 2H), 3.00 (m, 2H), 3.60 (s, 3H), 7.33-7.31 (m, 2H), 7.46 (t, J = 7.7 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.61 (dm, J = 7.9 Hz, 1H), 8.10 (dm, J = 7.9 Hz, 1H), 8.14 (m, 1H).

3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid

[0136] To a deoxygenated suspension of sodium ethanethiolate (0.135 g, 1.60 mmol) in DMF (0.5 mL) was added a solution of 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester (0.10 g, 0.32 mmol) in DMF (0.5 mL). Argon was bubbled through the mixture for 10 minutes. The reaction was heated at 100 °C for 1 hour and 200 °C for another hour. After the mixture cooled to room temperature, the reaction was quenched with 1 N HCl (20 mL) and was extracted with EtOAc (3 X 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to afford 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid (0.055 g, 57%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.51 (t, J = 8.0 Hz, 1H), 2.87-2.93 (m, 2H), 3.12-3.08 (m, 2H), 7.37 (m, 2H), 7.57-7.47 (m, 2H), 7.70 (dm, J = 7.9 Hz, 1H), 7.98 (dm, J = 7.8 Hz, 1H), 8.30 (m, 1H); ^{13}C NMR ($CDCl_3$) □26.2, 38.8, 128.1, 129.3, 129.7, 129.8, 129.9 (2C), 130.5, 133.3, 134.4, 137.7, 139.1, 141.2, 172.3, 176.9. Elemental analysis calculated for $C_{16}H_{14}O_4S$: C, 63.56; H, 4.67; S, 10.61. Found: C, 63.65; H, 4.88; S, 10.33.

EXAMPLE 2

Preparation of 2-[(4-carboxyphenyl)methoxy]-6-(2-`mercaptoethyl)- benzoic Acid (Scheme II)

5-Ethenyl-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one

[0137] A mixture of 2,2-dimethyl-5-trifluoromethanesulfonyloxy-4*H*-1,3-benzodioxin-4-one (9.90 g, 30.3 mmol), tributyl(vinyl)tin (10.10 g, 31.9 mmol), lithium chloride (8.70 g, 205 mmol), and triethylamine (5.0 mL, 36.0 mmol) in 1,4-dioxane (300 mL) was deoxygenated by bubbling nitrogen through the mixture for 1 hour. To the mixture was added tetrakis(triphenylphosphine)palladium (3.40 g, 2.90 mmol) and the mixture was heated at 100 °C for 3 hours. The mixture was allowed to cool to room temperature and was filtered. The filtrate was concentrated and purified by flash chromatography (1:12, EtOAc/hexanes) to provide 5-ethenyl-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one (5.00 g, 81%) as a yellow oil: ^{1}H NMR: ($CDCl_3$) δ 1.72 (s, 6H), 5.43 (dd, J = 11.0, 1.3 Hz, 1H), 5.72 (dd, J = 17.5, 1.3 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 7.27 (d, J = 8.0 Hz, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.73 (dd, J = 17.5, 11.0 Hz, 1H).

2-Ethenyl-6-hydroxybenzoic acid, methyl ester

[0138] To 5-ethenyl-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one (4.01 g, 19.6 mmol) was added 0.5 M sodium methoxide in methanol (85 mL, 42.5 mmol) at room temperature. Aqueous 1 N HCl (100 mL) was added to the solution after 15 minutes. The cloudy solution was extracted with ether (2 X 100 mL). The combined organic extracts were washed with H_2O (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered, and concentrated to afford 2-ethenyl-6-hydroxybenzoic acid, methyl ester (2.0 g, 57%) as a yellow oil. This material was used without further purification in the next step: ^{1}H NMR ($CDCl_3$) δ 3.96 (s, 3H), 5.26 (dd, J = 10.8, 1.5 Hz, 1H), 5.49 (dd, J = 17.3, 1.5 Hz, 1H), 6.95 (m, 2H), 7.23-7.39 (m, 2H), 11.12 (s, 1H).

2-Ethenyl-6-[4-(methoxycarbonyl)phenyl]methoxy-benzoic acid, methyl ester

[0139] To a stirred solution of the above material (0.500 g, 2.8 mmol) in acetone (10 mL) were added K_2CO_3 (1.50 g, 10.9 mmol) and methyl 4-(bromomethyl)benzoate (0.71 g, 3.10 mmol) at room temperature. The mixture was stirred under nitrogen for 3 hours and filtered. The filtrate was concentrated and residue was purified by flash chromatography (1:10

EtOAc/hexanes) to provide 2-ethenyl-6-[4-(methoxycarbonyl)phenyl]methoxy-benzoic acid, methyl ester (0.73 g, 80%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 6H), 5.17 (s, 2H), 5.37 (dd, J = 11.1, 1.0 Hz, 1H), 5.78 (dd, J = 17.6, 0.9 Hz, 1H), 6.70 (dd, 1, J = 17.4, 11.1 Hz, 1H), 6.83 (d, J = 7.8 Hz, 1H), 7.20 (d, J = 8.0 Hz, 1H) 7.29 (t, J = 8.0 Hz, 1H), 7.46 (d, J = 8.4 Hz, 2H), 8.04 (d, J = 8.3 Hz, 2H).

2-[2-(Acetylthio)ethyl]-6-[4-(methoxycarbonyl)phenyl]-methoxy-benzoic acid, methyl ester

[0140] To a solution of 2-ethenyl-6-[4-(methoxycarbonyl)phenyl]methoxy-benzoic acid, methyl ester (0.71 g, 2.18 mmol) in benzene (10 mL) was added thioacetic acid (1.80 mL, 25.2 mmol) followed by AIBN (37 mg, 0.23 mmol). After nitrogen was bubbled through the solution for 30 minutes, the solution was heated at reflux for 4 hours. The reaction was allowed to cool to room temperature and saturated $NaHCO_3$ (20 mL) was added. The mixture was extracted with EtOAc (3 X 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered and concentrated in *vacuo*. The residue was purified by flash chromatography (1:10 EtOAc/hexanes) to give 2-[2-(acetylthio)ethyl]-6-[4-(methoxycarbonyl)-phenyl]methoxy-benzoic acid, methyl ester (0.50 g, 60%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3H), 2.85-2.82 (m, 2H), 3.07-3.10 (m, 2H), 3.92 (s, 3H), 3.94 (s, 3H), 5.16 (s, 2H), 6.81 (d, J = 8.2 Hz, 1H), 6.92 (d, J = 7.7 Hz, 1H), 7.28 (t, J = 8.2 Hz, 1H), 7.46 (d, J = 8.3 Hz, 2H), 8.04 (d, J = 8.3 Hz, 2H).

2-[(4-Carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid

[0141] To a deoxygenated solution of 2-[2-(acetylthio)ethyl]-6-[4-(methoxycarbonyl)phenyl]methoxy-benzoic acid, methyl ester (0.20 g, 0.50 mmol) in 95% EtOH (3 mL) was added a deoxygenated solution of KOH (0.463 g, 8.3 mmol) in 95% EtOH (3 mL) under nitrogen. The solution was heated at reflux overnight and quenched by addition of 1 N HCl (20 mL). The mixture was extracted with EtOAc (3 x 20 mL) and the combined organic extracts were washed with water and brine, then dried over $MgSO_4$, filtered, and concentrated. Purification by flash chromatography (1:1 dichloromathane/hexanes with 1% acetic acid) provided 2-[(4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid (0.077 g, 46%) as a white solid: ^{1}H NMR (CD_3OD) δ 2.75 (m, 2H), 2.92 (m, 2H), 5.22 (s, 2H), 6.93 (d, J = 7.5 Hz, 1H), 6.98 (d, J = 8.2 Hz, 1H), 7.30 (t, J = 8.3 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 8.02 (d, J = 8.0 Hz, 2H); ^{13}C NMR (CD_3OD)

□26.5, 39.8, 71.1, 112.4, 123.9, 126.9, 128.3, 131.3, 131.7, 131.8, 139.9, 144.2, 156.7, 170.0, 172.3. Elemental analysis calculated for $C_{17}H_{16}O_5S$: C, 61.43; H, 4.85; S, 9.65. Found: C, 61.16; H, 4.95; S, 9.44.

EXAMPLE 3

Preparation of 4-mercaptomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid (Scheme III)

4-Methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid

[0142] To a solution of 2-bromo-5-methylbenzoic acid (5.00 g, 23.3 mmol) in DME (100 mL) were added 3-carboxyphenylboronic acid (3.86 g; 23.3 mmol), a solution of Na_2CO_3 (9.90 g, 93 mmol) in H_2O and tetrakis(triphenylphosphine)palladium. The mixture was stirred at 90 °C for 4 days. The mixture was allowed to cool to room temperature, diluted with EtOAc (50 mL), and washed with a saturated $NaHCO_3$ solution. The aqueous layer was separated, acidified with 10 % HCl, and extracted with EtOAc (3 X 20 mL). The combined extracts were dried over $MgSO_4$ and concentrated. The crude material was purified by column chromatography (9:1 hexanes/EtOAc 1% acetic acid) to afford 4-methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid (2.20 g, 37 %) as a solid: ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H), 7.30 (m, 1H), 7.42 (m, 1H), 7.52-7.57 (m, 2H), 7.60 (s, 1H), 7.85 (s, 1H), 7.91-7.92 (m, 1H).

4-Methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0143] To a solution of 4-methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid (2.20 g, 8.6 mmol) in methanol (150 mL) was added conc. H_2SO_4 (1.6 mL) and the mixture was heated at reflux overnight. The solvent was removed under a reduced pressure and the residue was partitioned between saturated aqueous $NaHCO_3$ solution and EtOAc (20 mL). The organic layer was dried over $MgSO_4$ and concentrated to give 4-methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (2.26 g, 92%) as a crude material. This product was used for the next reaction without further purification: ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H), 3.58 (s, 3H), 3.88 (s, 3H), 7.36-7.38 (m, 1H), 7.47-7.48 (m, 1H), 7.56-7.58 (m, 2H), 7.62 (s, 1H), 7.82 (s, 1H), 7.94-7.96 (m, 1H).

4-Bromomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0144] To a solution of 4-methyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (2.26 g, 7.9 mmol) in CCl_4 (50 mL) were added benzoyl peroxide (0.010 g, 0.04 mmol) and NBS (1.42 g, 8.0 mmol), and the mixture was refluxed for 3 days. The reaction mixture was allowed to cool to room temperature, filtered, and concentrated. The residue was purified by column chromatography (95:5 to 90:10 hexanes/EtOAc) to afford 4-bromomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.71 g, 60 %): ^{1}H NMR (DMSO-d_6) δ 3.61(s, 3H), 3.88 (s, 3H), 4.84 (s, 2H), 7.48-7.50 (d, J = 8.0 Hz, 1H), 7.59-7.60 (m, 2H), 7.72-7.75 (m, 1H), 7.85 (s, 1H), 7.90 (m, 1H), 7.97-7.99 (m, 1H).

4-Acetylthiomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0145] To a solution of 4-bromomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.59 g, 4.4 mmol) in acetone (75 mL) was added potassium thioacetate (0.60 g, 5.3 mmol), and the mixture was refluxed for 1 hour. The mixture was allowed to cool to room temperature, filtered, and concentrated. The residual product was purified by column chromatography (hexanes/EtOAc, 9/1) to afford 4-acetylthiomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.21 g, 76%): ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H), 3.60 (s, 3H), 3.88 (s, 3H), 4.23 (s, 2H), 7.42-7.44 (d, J = 8.0 Hz, 1H), 7.57-7.60 (m, 3H), 7.74 (s 1H), 7.83 (s, 1H), 7.96-7.99 (m, 1H).

4-Mercaptomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid

[0146] To a solution of 4-acetylthiomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.27 g, 0.75 mmol) in deoxygenated THF was added a degassed solution of sodium hydroxide (0.12 g, 3.0 mmol) in H_2O (5 mL) at room temperature. After 24 hours, additional solution of sodium hydroxide (0.09 g) in H_2O (2 mL) was added to the reaction mixture and the mixture was stirred for 24 hours. The mixture was acidified with 10 % HCl and extracted with EtOAc. The extract was dried over $MgSO_4$ and concentrated. The crude material was purified by column chromatography with (9:1 dichloromethane/EtOAc with 1% acetic acid) to afford 4-mercaptomethyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid (0.20 g, 92%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 3.10 (t, J = 8.03 Hz, 1H), 3.89 (d, J = 8.03 Hz, 2H), 7.43 (d, J = 7.53 Hz, 1H), 7.58-7.65 (m, 3H), 7.83 (d, J = 2.01 Hz, 1H), 7.92 (s, 1H), 7.97-8.00 (m, 1H). Elemental analysis calculated for $C_{15}H_{12}O_4S \cdot 0.5$ AcOH: C, 60.37; H, 4.43; O, 25.13; S, 10.07. Found: C, 60.28; H, 4.45; S, 10.15.

EXAMPLE 4

Preparation of 2-Carboxymethyl-6-(2-Mercaptoethyl)-Benzoic Acid)

(Scheme IV)

2-Hydroxy-6-methoxycarbonylmethyl-benzoic acid methyl ester

[0147] To a solution of 2-carboxymethyl-6-hydroxy-benzoic acid (5.021 g, 25.6 mmol) in DMF (100 mL) at 0 °C were added K_2CO_3 (3.567 g, 25.9 mmol) and CH_3I (7.9 mL, 51.9 mmol). After stirring under nitrogen at 0 °C for 4 h, the reaction was partitioned between H_2O (100 mL) and ether (150 mL). The aqueous layer was acidified with 1 N HCl and extracted with EtOAc (200 mL). The EtOAc layer was dried over $MgSO_4$ and concentrated in vacuo to give the crude 2-hydroxy-6-methoxycarbonylmethyl-benzoic acid methyl ester as a colorless oil (3.2 g, 56 %): ^{1}H NMR ($CDCl_3$) δ 3.69 (s, 3H), 3.89 (s, 5H), 6.72 (d, J = 6.7 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 7.33-7.38 (m, 1H).

2-Methoxycarbonylmethyl-6-trifluoromethanesulfonyloxy-benzoic acid methyl ester

[0148] To a solution of 2-hydroxy-6-methoxycarbonylmethyl-benzoic acid methyl ester (2.51 g, 11.2 mmol) in CH_2Cl_2 (50 mL) at 0 °C were added triflic anhydride (3.0 mL, 17.8 mmol) and pyridine (2.40 mL, 29.7 mmol). The solution was allowed to warm to rt overnight and was concentrated in vacuo. The residue was diluted with EtOAc (100 mL), washed with 1 N HCl (25 mL), saturated aqueous $NaHCO_3$ (25 mL), H_2O (25 mL), and brine (25 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (10% EtOAc/hexanes) to provide 2-methoxycarbonylmethyl-6-trifluoromethanesulfonyloxy-benzoic acid methyl ester as an oil (3.5 g, 90%): ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H), 4.06 (s, 2H), 4.10 (s, 3H), 7.4 (d, J = 7.6 Hz, 1H), 7.50 (d, J = 7.6 Hz, 1H), 7.67 (t, J = 8.2 Hz, 1H).

2-Methoxycarbonylmethyl-6-vinyl-benzoic acid methyl ester

[0149] A mixture of 2-methoxycarbonylmethyl-6-trifluoromethanesulfonyloxy-benzoic acid methyl ester (2.705 g, 7.6 mmol), LiCl (2.355 g, 55.6 mmol), NEt_3 (1.5 mL, 10.8 mmol), $Pd[P(C_6H_5)_3]_4$ (1.09 g, 0.94 mmol), and tributyl (vinyl)tin (2.85 mL, 9.75 mmol) in dioxane (50 mL) was heated at reflux. After 3 h, the reaction was allowed to cool to rt, filtered through a pad of silica gel and concentrated. The crude product was purified by flash chromatography on SiO_2 (13% EtOAc/hexanes) to afford 2-methoxycarbonylmethyl-6-vinyl-benzoic acid methyl ester as a yellow solid (1.50 g, 84%): ^{1}H NMR ($CDCl_3$) δ 3.67 (s,

3H), 3.89 (s, 5H), 5.32 (d, J = 10.9 Hz, 1H), 5.68 (d, J = 17.4 Hz, 1H), 6.82 (dd, J = 17.4, 10.9 Hz, 1H), 7.20 (d, J = 7.1 Hz, 1H), 7.36 (m, 1H), 7.50 (d, J = 7.8 Hz, 1H).

2-(2-Acetylsulfanyl-ethyl)-6-methoxycarbonylmethyl-benzoic acid methyl ester

[0150] Nitrogen was bubbled through a solution of 2-methoxycarbonylmethyl-6-vinyl-benzoic acid methyl ester (1.31 g, 5.60 mmol), thioacetic acid (4.00 mL, 56.0 mmol), and AIBN (0.184 g, 1.10 mmol) in benzene (30 mL) for 3 h at rt to remove oxygen. The mixture was heated at reflux for 3 h. The solution was allowed to cool to rt and saturated aqueous $NaHCO_3$ (100 mL) was added. The mixture was extracted with EtOAc (100 mL) and the organic layer was washed with H_2O (100 mL) and brine (100 mL), then dried over $MgSO_4$ and concentrated in vacuo. Flash chromatography on SiO_2 (10% EtOAc/hexanes) provided 2-(2-acetylsulfanyl-ethyl)-6-methoxycarbonylmethyl-benzoic acid methyl ester as a yellow solid (0.555 g, 33%): ^1H NMR ($CDCl_3$): δ 2.40 (s, 3H), 2.94-2.99 (m, 2H), 3.13-3.18 (m, 2H), 3.75 (s, 3H), 3.78 (s, 2H), 3.99 (s, 3H), 7.25 (d, J = 7.6 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H).

2-Carboxymethyl-6-(2-mercapto-ethyl)-benzoic acid (48)

[0151] A deoxygenated mixture of 2-(2-acetylsulfanyl-ethyl)-6-methoxycarbonylmethyl-benzoic acid methyl ester (0.555 g, 1.77 mmol) and 6 N KOH (3 mL) in EtOH (7 mL) was heated at reflux for 18 h. After allowing to cool to rt, the mixture was partitioned between 1 N HCl (75 mL) and EtOAc (100 mL). The organic layer was washed with H_2O (100 mL), brine (100 mL), dried over $MgSO_4$ and concentrated in vacuo. The crude product was dissolved in ether (100 mL), filtered to remove some unidentified solid material, and concentrated to give 2-carboxymethyl-6-(2-mercapto-ethyl)-benzoic acid as a white solid (0.310 g, 73%): ^1H NMR ($CDCl_3$) δ 1.37 (t, J = 8.2 Hz, 1H), 2.76 (m, 2H), 3.06 (m, 2H), 3.90 (s, 2H), 7.10 (d, J = 7.6 Hz, 1H), 7.21 (d, J = 6.9 Hz, 1H), 7.34 (t, J = 7.8 Hz, 1H): ^{13}C NMR ($CDCl_3$) □ 27.25, 40.43, 41.83, 131.58, 131.64, 132.45, 132.55, 134.66, 141.41, 176.14, 179.40. Elemental analysis calculated for $C_{11}H_{12}O_4S$: C, 54.99; H, 5.03; S, 13.35. Found: C, 55.20; H, 5.26; S, 13.32.

EXAMPLE 5

Preparation of Precursor Compound 4-[2-Carboxy-5-(Hydroxyamino)-5-Oxopentyl]Benzoic Acid

(Scheme XIII)

O-Benzyl-*N*-(2,4,6-trimethoxy-benzyl)hydroxylamine

[0152] 2,4,6-Trimethoxybenzaldehyde (12.42 g, 63.3 mmol) and *O*-benzylhydroxylamine (7.80 g, 63.3 mmol) were stirred in dichloromethane (65 mL) at rt overnight. The solvent was removed under reduced pressure. The crude residue was dissolved in ethanol (550 mL) and sodium cyanoborohydride (12.0 g, 189.9 mmol) was added dropwise. The mixture was stirred for 5-10 min, then 12 N HCl was added dropwise until pH became below 3. The reaction mixture was stirred for additional 3 h and 1 N NaOH was added until pH > 8. The product was extracted with dichloromethane. The organic phase was dried over Na_2SO_4 and concentrated under vacuo. The residue was purified by flash chromatography (5% ether in dichloromethane) to give 15.9 g of *O*-benzyl-*N*-(2,4,6-trimethoxy-benzyl)hydroxylamine as an oil (83% yield).

3-[Benzyloxy(2,4,6-trimethoxybenzyl)amino]-3-oxopropanoic acid

[0153] To a solution of *O*-benzyl-*N*-(2,4,6-trimethoxy-benzyl)hydroxylamine (2.0 g, 6.59 mmol) in dichloromethane (15 mL) was added triethylamine (1.0 mL, 7.25 mmol). The solution was cooled at 0 °C and ethyl malonyl chloride (0.85 mL, 6.59 mmol) was added. Precipitation was gradually formed. The resulting yellow slushy mixture was stirred at 0 °C for 15 min then brought up to rt and stirred for overnight. The solvent was removed under reduced pressure and the crude product was dissolved in EtOAc. The organic solution was consecutively washed with aq. 10% $KHSO_4$ and aq. saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated. The crude material was stirred in H_2O-THF (1:1 by volume, 30 mL) containing NaOH (1.32 g, 33 mmol) for 1 h. The reaction mixture was washed with EtOAc and the aqueous layer was acidified with aq. 10% $KHSO_4$ solution. The product was extracted with EtOAc, dried over Na_2SO_4, and concentrated to give 1.62 g of 3-[benzyloxy(2,4,6-trimethoxybenzyl)amino]-3-oxopropanoic acid as a white solid (63% yield).

N-Benzyloxy-3-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)-*N*-(2,4,6-trimethoxybenzyl)-propanamide

[0154] To a solution of 3-[benzyloxy(2,4,6-trimethoxybenzyl)amino]-3-oxopropanoic acid (9.41 g, 24.16 mmol), Meldrum's acid (3.84 g, 26.58 mmol), and DMAP (3.54 g, 29.0 mmol) in dichloromethane (125 mL) was dropwise added a solution of DCC (5.5 g, 26.6 mmol) in dichloromethane (25 mL) via addition funnel over a period of 1 h at 0 °C. The mixture was left in the refrigerator overnight. DCU was filtered off and the filtrate was washed with 5% $KHSO_4$ (3 times) and brine and dried over $MgSO_4$ for 4 h in the refrigerator. The drying agent was removed by filtration and acetic acid (16 mL) was added to the filtrate. The mixture was again cooled at 0 °C and sodium borohydride (2.3 g, 60.4 mmol) was added in small portions while stirring over 1 h. The reaction mixture was left overnight in the refrigerator. The next day, the solution was washed 3 times with brine and 2 times with water. The organic layer was concentrated in vacuo and the crude material was recrystallized from EtOAc-hexanes mixture to give 8.08 g of *N*-benzyloxy-3-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)-*N*-(2,4,6-trimethoxybenzyl)-propanamide as a white solid (67% yield).

5-{3-[Benzyloxy(2,4,6-trimethoxybenzyl)amino]-3-oxopropyl}-2,2-dimethyl-5-[4-(methoxycarbonyl)phenyl]methyl-[1,3]dioxane-4,6-dione

[0155] To stirring acetonitrile were added *N*-benzyloxy-3-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)-*N*-(2,4,6-trimethoxybenzyl)-propanamide (10.0 g, 19.9 mmol), methyl 4-(bromomethyl)benzoate (5.02 g, 21.9 mmol), potassium carbonate (4.125 g, 29.9 mmol), and benzyltriethylammonium chloride (6.799 g, 29.9 mmol). This reaction mixture was heated to 65 °C for 5h. The reaction mixture was then allowed to cool and extracted with 100 mL of 10% $KHSO_4$, 50 mL (3 times) of EtOAc, brine and then dried over Na_2SO_4. The crude product was purified by flash column chromatography (40% EtOAc in hexanes) to give 10.01 g of 5-{3-[benzyloxy(2,4,6-trimethoxybenzyl)amino]-3-oxopropyl}-2,2-dimethyl-5-[4-(methoxycarbonyl)phenyl]methyl-[1,3]dioxane-4,6-dione as a white powder (78% yield).

2-{3-[Benzyloxy(2,4,6-trimethoxybenzyl)amino]-3-oxopropyl}-2-(4-carboxyphenyl)methyl-malonic acid

[0156] To 5-{3-[benzyloxy(2,4,6-trimethoxybenzyl)amino]-3-oxopropyl}-2,2-dimethyl-5-[4-(methoxycarbonyl)phenyl]methyl-[1,3]dioxane-4,6-dione (5.38 g, 8.3 mmol) were added 50 mL of water, then 40 mL of 1,4-dioxane and finally a solution of NaOH (1.65 g, 41.4 mmol) in 20 mL of water. This mixture was heated at 100 °C for 2h. The solution was then allowed to cool to rt. The solvent was removed under reduced pressure and the residue was partitioned between 100 mL of 10% $KHSO_4$ and 100 mL of EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give 4.5 g of 2-{3-[benzyloxy(2,4,6-trimethoxybenzyl)amino]-3-oxopropyl}-2-(4-carboxyphenyl)-methylmalonic acid as an off white powder (95% yield).

4-[5-[Benzyloxy(2,4,6-trimethoxybenzyl)amino]-2-carboxy-5-oxopentyl]benzoic acid

[0157] 2-{3-[Benzyloxy(2,4,6-trimethoxybenzyl)amino]-3-oxopropyl}-2-(4-carboxyphenyl)methyl-malonic acid (9.30 g, 15.6 mmol) was taken up in DMSO (10 mL). This solution was heated at 130 °C for 1.5 h. The reaction mixture was then allowed to come to room temperature. The solvent was then removed under reduced pressure. EtOAc was used as an azeotrope to remove the DMSO. The residue was partitioned between 50 mL of 10% $KHSO_4$ and 100 mL of EtOAc. The organic layer was washed with brine (50 mL), dried over Na_2SO_4, and concentrated to give 6.17 g of 4-[5-[benzyloxy(2,4,6-trimethoxybenzyl)amino]-2-carboxy-5-oxopentyl]benzoic acid as an off white powder (76% yield). ^1H NMR (CDCl3) d 0.88-0.96 (m, 2H), 1.26-1.91 (m, 2H), 2.02-2.32 (m,2H), 2.47-2.83 (m, 2H), 3.01-3.60 (m,2H), 4.70 (s,1H), 7.14-7.29 (m,9H), 7.93-7.96 (d, 2H).

4-[5-(Benzyloxyamino)-2-carboxy-5-oxopentyl]benzoic acid

[0158] To stirring dichloromethane were added 4-[5-(benzyloxyamino)-2-carboxy-5-oxopentyl]benzoic acid (3.00 g, 5.4 mmol), then triisopropylsilane (0.855 g, 5.4 mmol) and TFA (4.7 mL).This mixture was stirred for 1.15 h. The solvent was then removed under reduced pressure. Dichloromethane (100 mL × 5) was used to azeotrope excess TFA. The residual solids were triturated with 1:1 EtOAc/hexanes containing 1.0 % AcOH to give 1.62 g of 4-[5-(benzyloxyamino)-2-carboxy-5-oxopentyl]benzoic acid as an off white colored powder (81% yield). ^1H NMR (MeOH-d6) δ 0.20-0.35 (m, 2H), 0.43-0.63 (m, 2H), 1.08-1.14 (m, 1H), 1.27-1.46 (m, 2H), 3.26 (s, 2H), 5.73-5.84 (m, 7H), 6.36-6.39 (d, 2H)

4-[2-Carboxy-5-(hydroxyamino)-5-oxopentyl]benzoic acid disodium salt

[0159] A 100-mL three neck round bottomed flask was charged with the 4-[5-(benzyloxyamino)-2-carboxy-5-oxopentyl]benzoic acid (0.500 g, 1.3 mmol), 0.5 N NaOH (5.2 mL, 2.6 mmol), water (8.3 ml), and a spatula full of 10 % Pd on carbon. The mixture was stirred under hydrogen (1 atm) for 7 h. The catalyst was removed by filtration and the filtrate was lyophilized to give 0.382 g of 4-[2-carboxy-5-(hydroxyamino)-5-oxopentyl]benzoic acid disodium salt as an off white powder (89% yield): ^{1}H NMR (D_2O) δ 1.75-1.90 (m, 2H), 2.10-2.30 (m, 2H), 2.45-2.60 (m, 1H), 2.75-3.00 (m, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.82 (d, J = 8.0 Hz, 2H). Elemental analysis calculated for $C_{13}H_{13}NNa_2O_6 \cdot 0.78H_2O$: C, 46.02; H, 4.33; N, 4.13. Found: C, 46.06; H, 4.14; N, 3.79.

EXAMPLE 6

Preparation of Precursor Compound 3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic Acid (Scheme XI)

3-(2,2-Dimethyl-4-oxo-4H-1,3-benzodioxin-5-yl)-benzoic acid, ethyl ester

[0160] To a solution of 2,2-dimethyl-5-trifluoromethanesulfonyloxy-4H-1,3-benzodioxin-4-one (2.0 g, 5.8 mmol), 3-ethoxycarbonylphenylboronic acid (1.34 g, 6.9 mmol) and anhydrous K2CO3 powder (2.61 g, 18.9 mmol) in DMF (30 mL) was added tetrakis(triphenylphosphine)palladium (0.202 g, 0.175 mmol). The mixture was heated at reflux for 2 h. The reaction mixture was allowed to cool to room temperature ("rt") and 1 N HCl (25 mL) was added. The mixture was extracted with EtOAc (3 x 25 mL). The combined extracts were washed with water and brine, then dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by flash chromatography (1:15 EtOAc/hexanes) to afford 3-(2,2-dimethyl-4-oxo-4H-1,3-benzodioxin-5-yl)-benzoic acid, ethyl ester (1.2 g, 63%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7.1 Hz, 3H), 1.80 (s, 6H), 4.39 (q, J = 7.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 2H), 7.47-7.57 (m, 3H), 8.00 (t, J = 1.5 Hz, 1H), 8.07 (dt, J = 7.5, 1.5 Hz, 1H).

3-Hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0161] To a solution of 3-(2,2-dimethyl-4-oxo-4H-1,3-benzodioxin-5-yl)benzoic acid, ethyl ester (1.4 g, 4.3 mmol) in methanol (10 mL) was added sodium methoxide (0.5 M in methanol, 25 mL) at 0 °C. The solution was stirred at rt for 15 min. The reaction was

quenched by addition of 1 N HCl (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic extracts were dried over MgSO4 and concentrated to afford 3-hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.2 g, 95%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 3H), 3.93 (s, 3H), 6.79 (dd, J = 7.5, 0.9 Hz, 1H), 7.04 (dd, J = 7.5, 0.9 Hz, 1H), 7.43 (m, 3H), 7.93 (m, 1H), 8.02 (dm, J = 7.0 Hz, 1H), 10.8 (s, 1H).

3-Trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0162] To a solution of 3-hydroxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.1 g, 3.8 mmol) in dichloromethane (15 mL) were added pyridine (1.00 mL, 12.3 mmol) and trifluromethanesulfonic anhydride (0.90 mL, 5.4 mmol) at 0 °C. The solution was stirred at 0 °C for 2 h. Aqueous 1 N HCl (20 mL) was added, and the mixture was extracted with dichloromethane (3 x 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to give 3-trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.4 g, 87%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 3.72 (s, 3H), 3.94 (s, 3H), 7.38-7.62 (m, 5H), 8.08 (m, 2H).

3-Ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0163] A mixture of 3-trifluoromethanesulfonyloxy-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (1.3 g, 3.1 mmol), tetrakis(triphenylphosphine)palladium (0.36 g, 0.31 mmol), LiCl (0.94 g, 22.2 mmol), triethylamine (0.6 mL, 4.3 mmol) and tri-n-butyl(vinyl)tin (1.0 mL, 3.4 mmol) in 1,4-dioxane (30 mL) was heated at reflux under N_2 for 4 h. After cooling to rt, the mixture was filtered through a plug of silica gel and the filtrate was concentrated. Purification by flash chromatography (1:10 EtOAc/hexanes) provided 3-ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.91 g, 99%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 3H), 3.92 (s, 3H), 5.40 (d, J = 11.1 Hz, 1H), 5.79 (d, J = 17.5 Hz, 1H), 6.87 (dd, J = 17.4, 11.0 Hz, 1H), 7.31 (d, J = 7.5 Hz, 1H), 7.44-7.49 (m, 2H), 7.56 (dm, J = 7.5 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 8.03 (dm, J = 7.5 Hz, 1H), 8.08 (t, 1, J = 1.5 Hz, 1H).

3-[2-(Acetylthio)ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester

[0164] To a solution of 3-ethenyl-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.85 g, 2.9 mmol) in benzene (10 mL) was added thioacetic acid (2.1 mL, 29.4 mmol) followed by AIBN (0.053 g, 0.32 mmol). The solution was deoxygenated for 30 min by

bubbling nitrogen through the solution and then heated at reflux for 4 h. Saturated aqueous NaHCO3 (20 mL) was added to the solution and the mixture was extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (1:12 EtOAc/hexanes) to give 3-[2-(acetylthio)ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.51 g, 48%) as an off white solid: ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H), 2.93 (m, 2H), 3.14 (m, 2H), 3.62 (s, 3H), 3.93 (s, 3H), 7.29 (dd, J = 7.6, 0.9 Hz, 1H), 7.35 (dd, J = 7.5, 0.8 Hz, 1H), 7.44 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.55 (dt, J = 8.0, 1.5 Hz, 1H), 8.03 (dt, J = 7.9, 1.5 Hz, 1H), 8.07 (t, J = 1.5 Hz, 1H).

3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester

[0165] To a deoxygenated solution of 3-[2-(acetylthio)ethyl]-[1,1'-biphenyl]-2,3'-dicarboxylic acid, dimethyl ester (0.50 g, 1.34 mmol) in THF (3.5 mL) was added a deoxygenated solution of NaOH (0.38 g, 9.4 mmol) in water (3.5 mL). The mixture was stirred overnight, and 1 N HCl (20 mL) was added. The mixture was extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to afford 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester (0.35 g, 83%) as an off white solid: ^{1}H NMR ($CDCl_3$) δ 1.46 (t, J = 8.0 Hz, 1H), 2.83 (m, 2H), 3.00 (m, 2H), 3.60 (s, 3H), 7.33-7.31 (m, 2H), 7.46 (t, J = 7.7 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.61 (dm, J = 7.9 Hz, 1H), 8.10 (dm, J = 7.9 Hz, 1H), 8.14 (m, 1H).

3-(2-Mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid

[0166] To a deoxygenated suspension of sodium ethanethiolate (0.135 g, 1.60 mmol) in DMF (0.5 mL) was added a solution of 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid, 2-methyl ester (0.10 g, 0.32 mmol) in DMF (0.5 mL). Argon was bubbled through the mixture for 10 min. The reaction was heated at 100 °C for 1 h and 200 °C for another hour. After the mixture cooled to rt, the reaction was quenched with 1 N HCl (20 mL) and was extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to afford 3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid (0.055 g, 57%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.51 (t, J = 8.0 Hz, 1H), 2.87-2.93 (m, 2H), 3.12-3.08 (m, 2H), 7.37 (m, 2H), 7.57-7.47 (m, 2H), 7.70 (dm, J = 7.9 Hz, 1H), 7.98 (dm, J = 7.8 Hz, 1H), 8.30 (m, 1H);

^{13}C NMR ($CDCl_3$) δ 26.2, 38.8, 128.1, 129.3, 129.7, 129.8, 129.9 (2C), 130.5, 133.3, 134.4, 137.7, 139.1, 141.2, 172.3, 176.9. Elemental analysis calculated for $C_{16}H_{14}O_4S$: C, 63.56; H, 4.67; S, 10.61. Found: C, 63.65; H, 4.88; S, 10.33.

EXAMPLE 7

In Vitro Inhibition of NAALADase Activity

[0167] Various compounds used in the inventive methods and pharmaceutical compositions have been tested for *in vitro* inhibition of NAALADase activity. The experimental protocol and some of the results are set forth in U.S. Patents Nos. 5,672,592, 5,795,877, 5,863,536, 5,880,112, 5,902,817, 5,962,521, 5,968,915, 6,025,344, 6,025,345, 6,028,216, 6,046,180, 6,054,444, 6,071,965, 6,121,252, 6,265,609, 6,348,464, 6,452,044, 6,458,775, 6,586,623, and International Publications Nos. WO 01/14390, WO 02/096866, WO 03/057670 and WO 02/092553, the entire contents of which patents and publications are herein incorporated by reference, as though set forth herein in full.

[0168] Other results are provided below in TABLES II and III.

TABLE II

IN VITRO INHIBITION OF NAALADASE ACTIVITY

Compound	K_i (nM)
4-[4-(2,4-dicarboxybenzoyl)phenoxy]-1,2-benzenedicarboxylic acid	1170
2-[(4-carboxyphenyl)sulfonyl]-1,4-benzenedicarboxylic acid	2370
2-[(2,5-dicarboxyphenyl)sulfonyl]-1,4-benzenedicarboxylic acid	1870
4-[(2-carboxyphenyl)thio]-1,3-benzenedicarboxylic acid	3980
2-[(2-carboxyphenyl)thio]-1,4-benzenedicarboxylic acid	572
4-[3-[[3-(2,4-dicarboxyphenoxy)-propyl]-dithio]propoxy]-1,3-benzenedicarboxylic acid	3750
5-(3-mercaptopropoxy)-1,3-benzenedicarboxylic acid	3300
5-(2-mercaptoethoxy)-1,3-benzenedicarboxylic acid	14500
5-[(hydroxyamino)-carbonyl]-1,3-benzenedicarboxylic acid	1000
5-phosphono-1,3-benzenedicarboxylic acid	14000
5-mercaptomethyl-1,3-benzenedicarboxylic acid	6500
5-phosphonomethyl-1,3-benzenedicarboxylic acid	3100
5-[(carboxymethyl)amino]-1,3-benzenedicarboxylic acid	100000
5-[[(2-furanylmethyl)amino]methyl]-1,3-benzenedicarboxylic acid	50000
2-carboxymethyl-1,4-benzenedicarboxylic acid	9000
5-[2-(hydroxyamino)-2-oxoethyl]-1,3-benzenedicarboxylic	12000

Compound	K_i (nM)
acid	
4-(2-mercaptoethyl)-1,3-benzenedicarboxylic acid	116
5-(2-mercaptoethyl)-1,3-benzenedicarboxylic acid	5100

TABLE III

***IN VITRO* INHIBITION OF NAALADASE ACTIVITY**

Compound	IC_{50}
alpha-(3-mercaptopropyl)-3-(trifluoromethyl)-benzenepropanoic acid	4000
alpha-(3-mercaptopropyl)-benzenepropanoic acid	2270
4-hydroxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	2140
2,3,4,5,6-pentafluoro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	1000
3-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	17
4-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	59
alpha-(3-mercaptopropyl)-4-(methylsulfonyl)-benzenepropanoic acid	211
2-cyano-alpha-(3-mercaptopropyl)-benzenepropanoic acid	450

Compound	IC_{50}
5-(2-carboxy-5-mercaptopentyl)-1,3-benzenedicarboxylic acid	2.55
5-carboxy-2-chloro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	2.09
3-carboxy-4-fluoro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	12
4-(2-cyanophenyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	316
2-(aminocarbonyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	3950
3-(1-carboxy-4-mercaptobutoxy)-benzoic acid	16.3
5-mercapto-2-phenoxy-pentanoic acid	555
2-(3,5-dimethoxyphenoxy)-5-mercapto-pentanoic acid	16,100
alpha-(3-mercaptopropyl)-2,5-dimethoxy-benzenepropanoic acid	566
alpha-(3-mercaptopropyl)-3-phenoxy-benzenepropanoic acid	308
2-(3-hydroxyphenoxy)-5-mercapto-pentanoic acid	846
3-(1-carboxy-4-mercaptobutoxy)-benzeneacetic acid	64
4-(1-carboxy-4-mercaptobutoxy)-benzeneacetic acid	82
alpha-(3-mercaptopropyl)-4-phenyl-benzenepropanoic acid	229

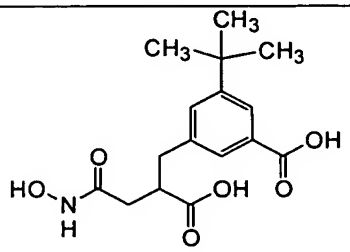
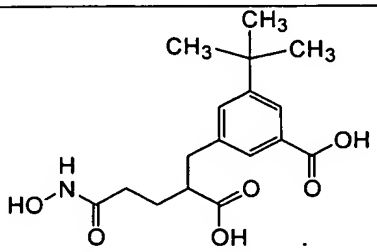
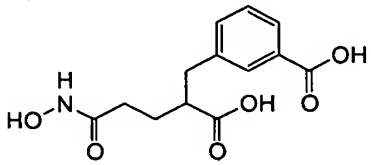
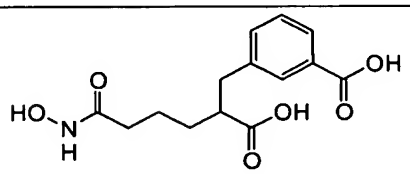
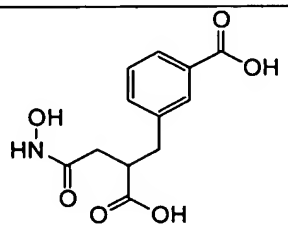
Compound	IC_{50}
2-(3-acetylphenoxy)-5-mercapto-pentanoic acid	2900
2-[3-(acetylamino)phenoxy]-5-mercapto-pentanoic acid	27700
2-(4-acetylphenoxy)-5-mercaptopentanoic acid	29.8
4-(acetylamino)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	2200
3-(1-carboxy-4-mercaptobutoxy)-4-methoxy-benzoic acid	287
4-(carboxymethyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	809
2-(1-carboxy-4-mercaptobutoxy)-benzoic acid	4600
4-(1-carboxy-4-mercaptobutoxy)-benzoic acid	192
3-carboxy-2-chloro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	1200
3-carboxy-4-chloro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	175
3-(1-carboxy-4-mercaptobutoxy)-4-chloro-benzoic acid	118
3-(1-carboxy-4-mercaptobutoxy)-4-fluoro-benzoic acid	400
5-carboxy-2-fluoro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	6.33
5-carboxy-alpha-(3-mercaptopropyl)-2-methoxy-benzenepropanoic acid	26

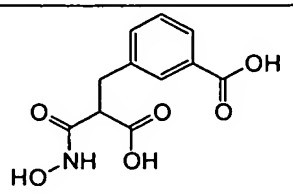
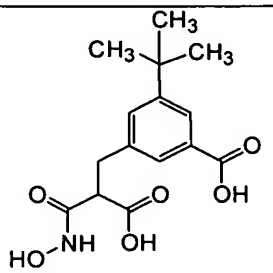
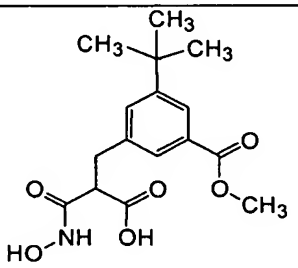
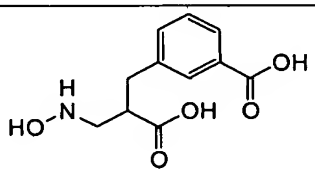
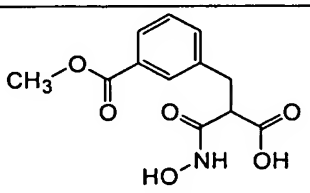
Compound	IC_{50}
4-carboxy-alpha-(3-mercaptopropyl)-1-naphthalenepropanoic acid	316
2-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	1700
4-carboxy-2,3,5,6-tetrafluoro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	660
5-mercapto-2-(phenylthio)-pentanoic acid	297
3-[1-carboxy-4-mercaptobutyl)thio]-benzoic acid	24
alpha-(3-mercaptopropyl)-2-naphthalenepropanoic acid	1130
2-chloro-alpha-(3-mercaptopropyl)-benzenepropanoic acid	1350
alpha-(3-mercaptopropyl)-3-[[(phenylmethyl)amino]carbonyl]-benzenepropanoic acid	1130
3-bromo-5-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	2.5
3-[[(carboxymethyl)amino]carbonyl]-alpha-(3-mercaptopropyl)-benzenepropanoic acid	286
3-bromo-4-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	146
3-carboxy-alpha-(3-mercaptopropyl)-5-nitro-benzenepropanoic acid	3

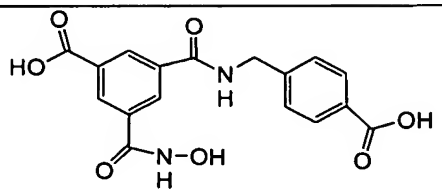
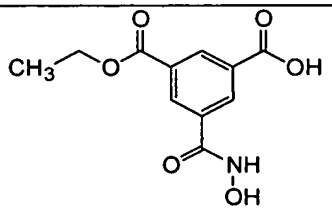
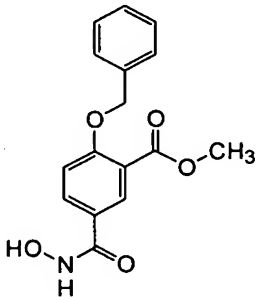
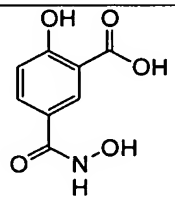
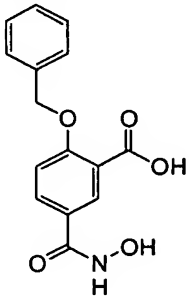
Compound	IC_{50}
3-carboxy-5-(1,1-dimethylethyl)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	0.05
5-carboxy-alpha-(3-mercaptopropyl)-2-nitro-benzenepropanoic acid	8.25
3'-(2-carboxy-5-mercaptopentyl)-[1,1'-biphenyl]-3-carboxylic acid	1.83
2-bromo-5-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	3.33
(+)-3-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	7
(-)-3-carboxy-alpha-(3-mercaptopropyl)-benzenepropanoic acid	33.3
2-(2-carboxy-5-mercaptopentyl)-[1,1'-biphenyl]-4-carboxylic acid	19
6-(2-carboxy-5-mercaptopentyl)-[1,1'-biphenyl]-2-carboxylic acid	70
4-(2-carboxy-5-mercaptopentyl)-[1,1'-biphenyl]-2-carboxylic acid	18
3-carboxy-alpha-(3-mercaptopropyl)-5-methoxy-benzenepropanoic acid	13
3'-(2-carboxy-5-mercaptopentyl)-[1,1'-biphenyl]-2-carboxylic acid	184

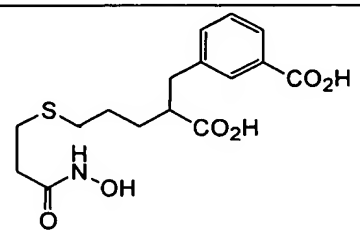
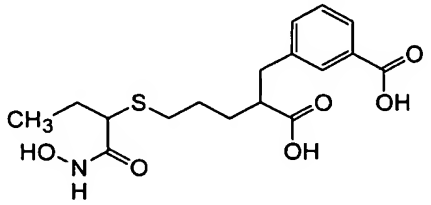
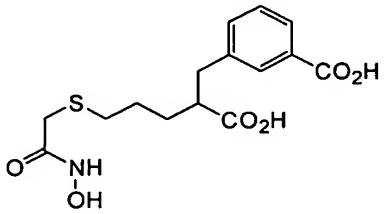
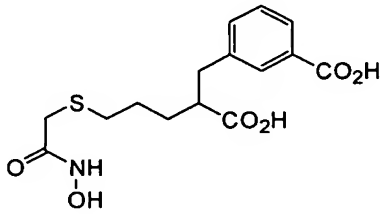
Compound	IC_{50}
3-(2-carboxyphenoxy)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	49
4-(2-carboxyphenoxy)-alpha-(3-mercaptopropyl)-benzenepropanoic acid	3590
3-carboxy-alpha-(3-mercaptobutyl)-benzenepropanoic acid	30
4'-(2-carboxy-5-mercaptopropyl)-[1,1'-biphenyl]-2-carboxylic acid	87
alpha-(3-mercaptopropyl)-3-phenyl-benzenepropanoic acid	181
3-carboxy-alpha-(3-mercaptopropyl)-5-phenoxy-benzenepropanoic acid	5
3-carboxy-5-(1,1-dimethylethyl)-alpha-(3-mercaptobutyl)-benzenepropanoic acid	1
2-(2-mercaptoethyl)-benzoic acid	613
5-hydroxy-2-(2-mercaptoethyl)-benzoic acid	9170
5-[(4-carboxyphenyl)methoxy]-2-(2-mercaptoethyl)-benzoic acid	71.5
2-(2-mercaptoethyl)-5-(phenylmethoxy)-benzoic acid,	380
2-(carboxymethoxy)-6-(2-mercaptoethyl)-benzoic acid	215
5-[(3-carboxyphenyl)methoxy]-2-(2-mercaptoethyl)-benzoic acid	84.5
2-(2-mercaptoethyl)-6-(phenylmethoxy)-benzoic acid	11.5
2-[(2-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	11.5
2-[(4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-	4

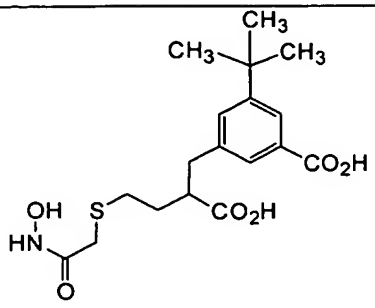
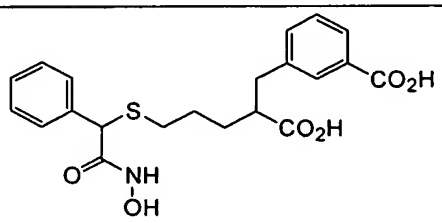
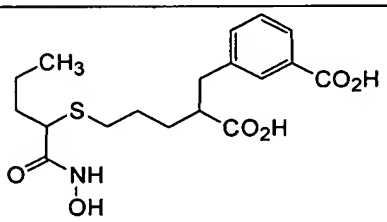
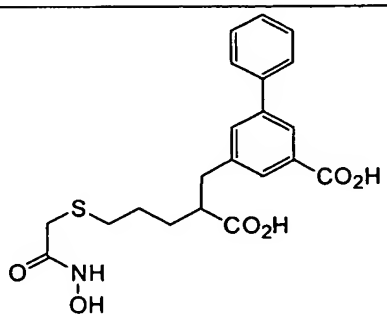
Compound	IC_{50}
benzoic acid	
3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid	3.46
5-(mercaptomethyl)-2-(2-phenylethoxy)-benzoic acid	188
2-(3,3-dimethylbutoxy)-6-(2-mercaptoethyl)-benzoic acid	2580
2-(2-mercaptoethyl)-6-(2-phenylethoxy)-benzoic acid	266
2-[(2-chlorophenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	160
2-[[3-carboxy-5-(1,1-dimethylethyl)phenyl]methoxy]-6-(2-mercaptoethyl)-benzoic acid	23.7
3-(2-mercaptoethyl)-[1,1'-biphenyl]-2,4'-dicarboxylic acid	4.28
2-[(4-carboxy-2-methoxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	7
2-[(4-carboxy-3-methoxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	10.5
2-[(2-bromo-4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	2.65
2-[(3-bromo-4-carboxyphenyl)methoxy]-6-(2-mercaptoethyl)-benzoic acid	0.098
2-(2-mercaptoethyl)-6-phenoxy-benzoic acid	1150
4-(mercaptomethyl)-[1,1'-biphenyl]-2,3'-dicarboxylic acid	76
5-(mercaptomethyl)-2-(phenylmethoxy)-benzoic acid	85
4-bromo-3-(mercaptomethyl)-benzoic acid	3200
3-(2-mercaptoethyl)-benzoic acid	6050
3-(mercaptomethyl)-benzoic acid	3780
2-(mercaptomethyl)-benzoic acid	100000

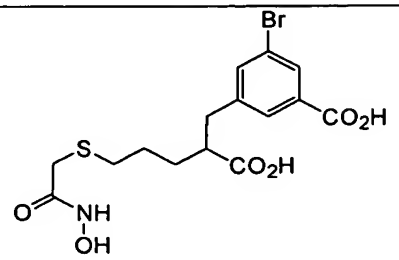
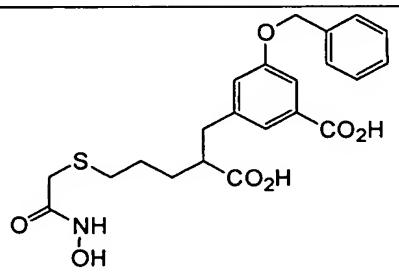
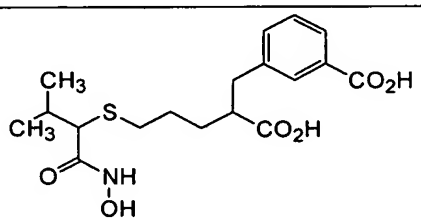
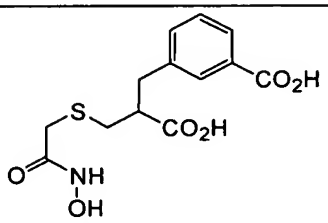
Compound	IC_{50}
 3-*tert*-Butyl-5-(2-carboxy-3-hydroxycarbamoyl-propyl)-benzoic acid	296.3
 3-*tert*-Butyl-5-(2-carboxy-4-hydroxycarbamoyl-butyl)-benzoic acid	0.2
 3-(2-Carboxy-4-hydroxycarbamoyl-butyl)-benzoic acid	47.8
 3-(2-Carboxy-5-hydroxycarbamoyl-pentyl)-benzoic acid	66.3
 3-(2-Carboxy-3-hydroxycarbamoyl-propyl)-benzoic acid	693

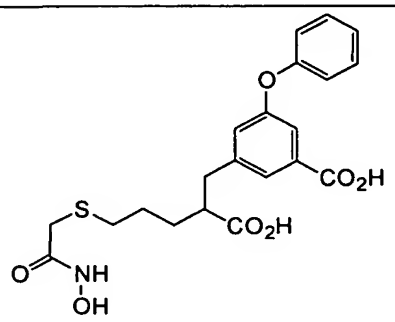
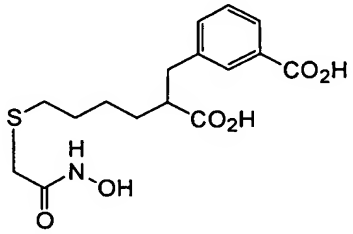
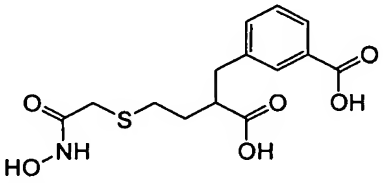
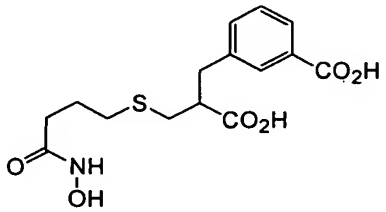
Compound	IC_{50}
 3-(2-Carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid	4530
 3-*tert*-Butyl-5-(2-carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid	11900
 3-*tert*-Butyl-5-(2-carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid methyl ester	38600
 3-(2-Carboxy-3-hydroxyamino-propyl)-benzoic acid	44000
 3-(2-Carboxy-2-hydroxycarbamoyl-ethyl)-benzoic acid methyl ester	100000

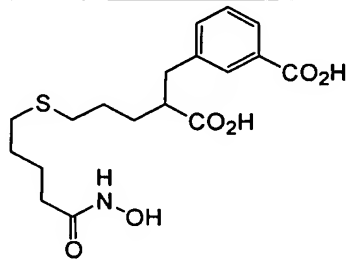
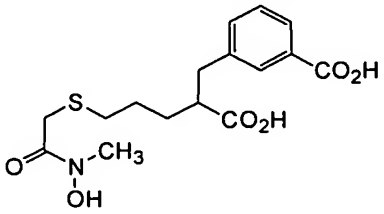
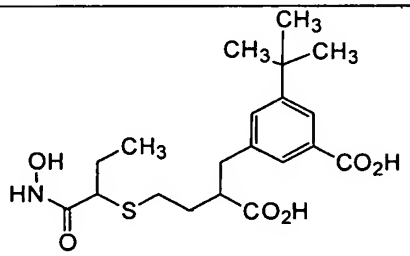
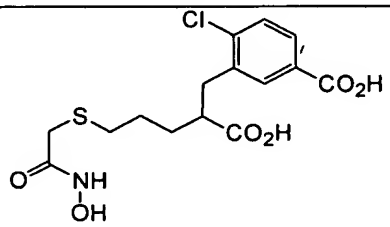
Compound	IC_{50}
	2780
 5-Hydroxycarbamoyl-isophthalic acid monoethyl ester	29000
 6-Benzyloxy-N-hydroxy-isophthalamic acid methyl ester	100000
 6,N-Dihydroxy-isophthalamic acid	100000
 6-Benzyloxy-N-hydroxy-isophthalamic acid	100000

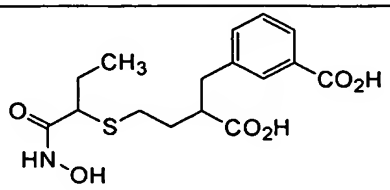
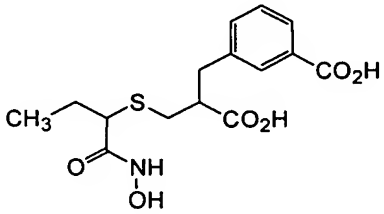
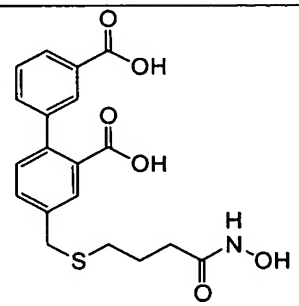
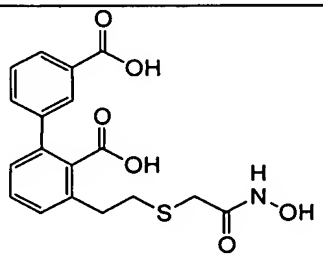
Compound	IC_{50}
 3-[2-Carboxy-5-(2-hydroxycarbamoyl-ethylsulfanyl)-pentyl]-benzoic acid	113
 3-[2-Carboxy-5-(1-hydroxycarbamoyl-propylsulfanyl)-pentyl]-benzoic acid	3291
 3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid	384
 3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanyl-pentyl)-benzoic acid	394

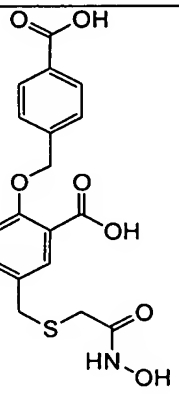
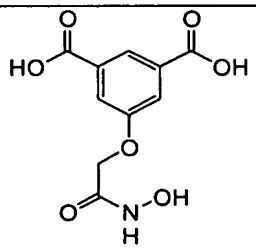
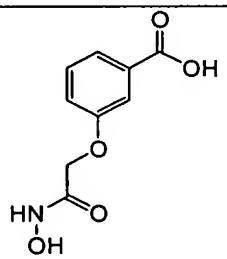
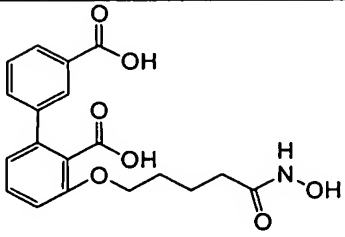
Compound	IC_{50}
 3-*tert*-Butyl-5-(2-carboxy-4-hydroxycarbamoylmethyl-sulfanylbutyl)-benzoic acid	1060
 3-[2-Carboxy-5-(hydroxycarbamoyl-phenyl-methylsulfanyl)-pentyl]-benzoic acid	1120
 3-[2-Carboxy-5-(1-hydroxycarbamoyl-butylsulfanyl)-pentyl]-benzoic acid	1530
 5-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-biphenyl-3-carboxylic acid	1690

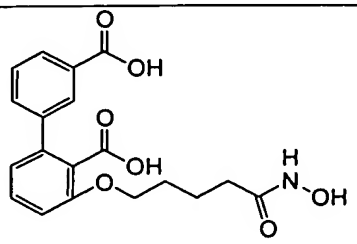
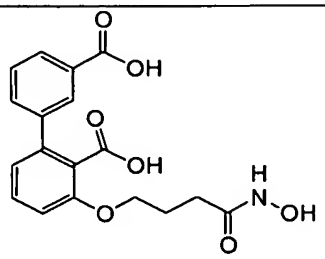
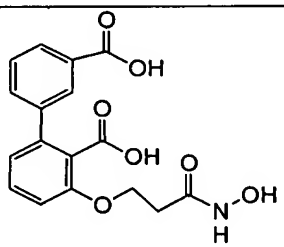
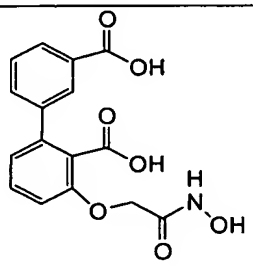
Compound	IC_{50}
 3-Bromo-5-(2-carboxy-5-hydroxycarbamoyl-methylsulfanylpentyl)-benzoic acid	2460
 3-Benzyloxy-5-(2-carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-benzoic acid	2570
 3-[2-Carboxy-5-(1-hydroxycarbamoyl-2-methyl-propylsulfanyl)-pentyl]-benzoic acid	2800
 3-(2-Carboxy-3-hydroxycarbamoylmethyl-sulfanylpropyl)-benzoic acid	3070

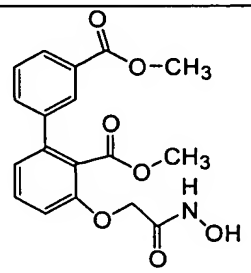
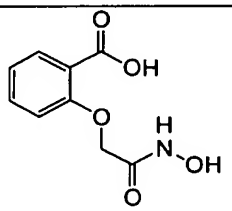
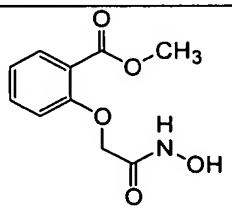
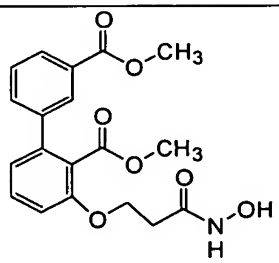
Compound	IC_{50}
 3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-5-phenoxy-benzoic acid	3250
 3-(2-Carboxy-6-hydroxycarbamoylmethyl-sulfanylhexyl)-benzoic acid	3330
 3-(2-Carboxy-4-hydroxycarbamoylmethyl-sulfanylbutyl)-benzoic acid	4900
 3-[2-Carboxy-3-(3-hydroxycarbamoyl-propylsulfanyl)-propyl]-benzoic acid	6050

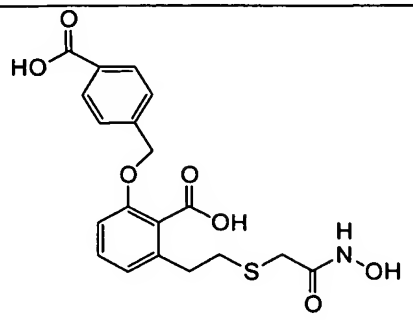
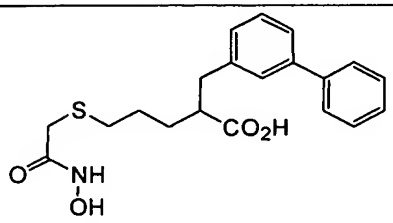
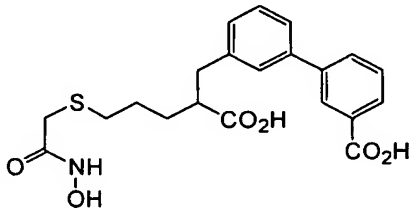
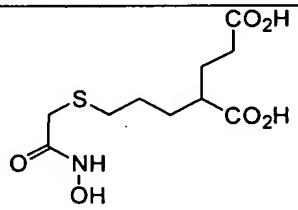
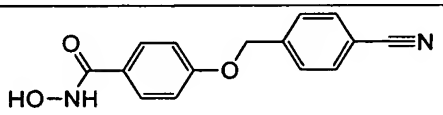
Compound	IC_{50}
 3-[2-Carboxy-5-(4-hydroxycarbamoyl-butylsulfanyl)-pentyl]-benzoic acid	7580
 3-{2-Carboxy-5-[(hydroxy-methyl-carbamoyl)-methylsulfanyl]-pentyl}-benzoic acid	14900
 3-*tert*-Butyl-5-[2-carboxy-4-(1-hydroxycarbamoyl-propylsulfanyl)-butyl]-benzoic acid	16000
 3-(2-Carboxy-5-hydroxycarbamoylmethyl-sulfanylpentyl)-4-chloro-benzoic acid	16000

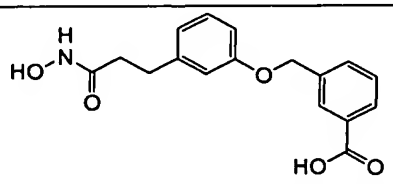
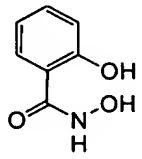
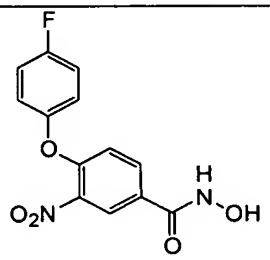
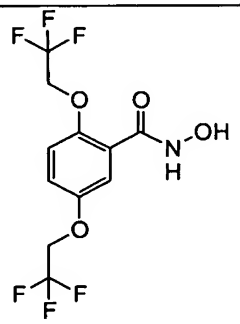
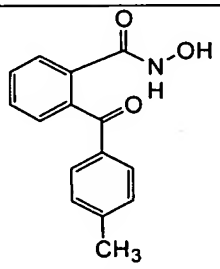
Compound	IC_{50}
 3-[2-Carboxy-4-(1-hydroxycarbamoyl-propylsulfanyl)-butyl]-benzoic acid	19000
 3-[2-Carboxy-3-(1-hydroxycarbamoyl-propylsulfanyl)-propyl]-benzoic acid	41500
 4-(3-Hydroxycarbamoyl-propylsulfanylmethyl)-biphenyl-2,3'-dicarboxylic acid	10820
 3-(2-Hydroxycarbamoyl-methylsulfanyl-ethyl)-biphenyl-2,3'-dicarboxylic acid	255

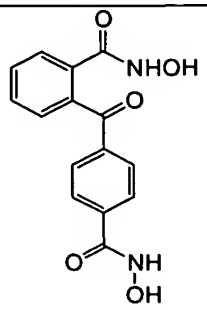
Compound	IC_{50}
	1330
 5-Hydroxycarbamoylmethoxy-isophthalic acid	14800
 3-Hydroxycarbamoylmethoxy-benzoic acid	40800
 3-(4-Hydroxycarbamoyl-butoxy)-biphenyl-2,3'-dicarboxylic acid	55.9

Compound	IC_{50}
 3-(4-Hydroxycarbamoyl-butoxy)-biphenyl-2,3'-dicarboxylic acid	4800
 3-(3-Hydroxycarbamoyl-propoxy)-biphenyl-2,3'-dicarboxylic acid	9100
 3-(2-Hydroxycarbamoyl-ethoxy)-biphenyl-2,3'-dicarboxylic acid	14900
 3-Hydroxycarbamoylmethoxy-biphenyl-2,3'-dicarboxylic acid	27600

Compound	IC_{50}
 3-Hydroxycarbamoylmethoxy-biphenyl-2,3'-dicarboxylic acid dimethyl ester	100000
 2-Hydroxycarbamoylmethoxy-benzoic acid	100000
 2-Hydroxycarbamoylmethoxy-benzoic acid methyl ester	100000
 3-(2-Hydroxycarbamoyl-ethoxy)-biphenyl-2,3'-dicarboxylic acid dimethyl ester	100000

Compound	IC_{50}
	14000
 2-Biphenyl-3-ylmethyl-5-hydroxy-carbamoylmethylsulfanyl-pentanoic acid	4210
 3'-(2-Carboxy-5-hydroxycarbamoylmethylsulfanyl-pentyl)-biphenyl-3-carboxylic acid	23500
 2-(3-Hydroxycarbamoyl-methylsulfanylpropyl)-pentanedioic acid	3000
 4-(4-Cyano-benzyloxy)-N-hydroxy-benzamide	14150

Compound	IC_{50}
 3-[3-(2-Hydroxycarbamoyl-ethyl)-phenoxymethyl]-benzoic acid	92675
 2,N-Dihydroxy-benzamide	100000
 4-(4-Fluoro-phenoxy)-N-hydroxy-3-nitro-benzamide	100000
 N-Hydroxy-2,5-bis-(2,2,2-trifluoro-ethoxy)-benzamide	100000
 N-Hydroxy-2-(4-methyl-benzoyl)-benzamide	100000

Compound	IC_{50}
	100000

EXAMPLE 8

Effects of NAALADase Inhibitors on Morphine Tolerance (Experiment 1) and Acute Effects in the Tail-Flick Test (Experiment 2)

EXPERIMENTAL PROTOCOL

Subjects

[0169] Male C57/BL mice (IMP, Lodz, Poland), 22-24 g of body weight were group-housed in the standard laboratory cages and kept in a temperature-controlled colony room (21 ± 2°C) with a 12-hr light/dark cycle (light on: 07:00, off: 19:00). Commercial food and tap water were available *ad libitum*. Each experimental group consisted of 7–28 mice per treatment. All mice were used only once.

Apparatus for Experiments 1-2

[0170] A standardized tail-flick analgesia meter (Columbus, Ohio, USA, model 33), adjusted to sensitivity of "10" with radiant heat source and connected to an automatic timer was used to assess antinociceptive responses. The intensity of the heat stimulus was adjusted so that the baseline tail-flick latency was ~ 3 s. A maximum latency of 10 s (i.e., cut-off) was used to minimize damage to the tail. The tail withdrawal latency was measured from the start of heat stimulus until the mouse exhibited a flick of the tail. Each response assessment consisted of two separate measurements taken at different portions of the tail (spaced by 1.5-2 cm) and separated by 15 s. The mean of these responses was used for subsequent comparisons.

[0171] Morphine antinociceptive potency was investigated with the use of cumulative dose-response curves that allowed for minimization of the animal number used (Paronis and Holtzman 1991). After adaptation and baseline trials, each mouse was injected s.c. with a low dose of morphine (1 mg/kg). Thirty min later, the mouse was retested and injected with the next dose of morphine that was increased by quarter of a log unit. Thus, because the initial dose of morphine was 1.0 mg/kg, the next dose was 1.78 mg/kg, for a cumulative dose of 2.8 mg/kg. This procedure continued until either the mouse did not move his tail within the cut-off time or until there was a plateauing of the dose-response curve, so that the latency did not increase from one dose to the next. Each analgesic responder was not subjected to further tail flick assessments but was injected with the subsequent dose of morphine so that every animal received the same total dose of morphine during a given test.

Effects on Morphine Tolerance (Experiment 1) and Acute Effects in the Tail-Flick Test (Experiment 2)

[0172] Experiment 1 was carried out to investigate the effect of 2-PMPA on the development of morphine tolerance. On day 1 (test #1), the first measurement of morphine antinociceptive potency was performed, followed by 6 days of bid morphine injections (10 mg/kg, s.c., 7:30 and 17:30) (Elliott et al. 1994; Popik et al. 2000b). Pretreatment with 2-PMPA (30, 50 or 100 mg/kg, i.p.) or memantine (7.5 mg/kg, s.c., a "positive control") was given at 30 min prior to each morphine dose on days 2-7. On day 8 (test #2), the second measurement of morphine antinociceptive potency was carried out. The degree of morphine tolerance was assessed by comparing the morphine antinociceptive potencies (cumulative dose-response curves) obtained in tests #1 and #2.

[0173] Experiment 2 was designed to determine whether 2-PMPA might itself produce antinociceptive effects and/or affect the antinociceptive effects of morphine. Morphine (1.5 or 3 mg/kg, s.c.) was administered 30 min after injection of 100 mg/kg of 2-PMPA or placebo, administered i.p. The 3 mg/kg dose of morphine corresponds to the antinociceptive ED_{50} dose in these test conditions (data not shown).

RESULTS

Effects of 2-PMPA on Development of Morphine Tolerance (Experiment 1)

[0174] There were no differences in antinociceptive morphine ED_{50} values on test #1 among groups (Table IV). Treatment with 10 mg/kg bid of morphine produced 6.44 fold increase in the ED_{50} values as determined on test #2. In contrast, pretreatment with memantine, 50 or 100 (but not 30) mg/kg of 2-PMPA given prior to each dose of morphine attenuated the development of morphine tolerance. The effects of 2-PMPA were related to the dose. This was evidenced by a significant decrease in both test #2 ED_{50} values (statistically significant for the dose 100 mg/kg) and antinociceptive morphine fold shifts of 2-PMPA for the doses of 100 and 50 mg/kg, as compared with the control group that received placebo+morphine (Table IV). Similarly, memantine (7.5 mg/kg) produced an inhibition of morphine tolerance.

TABLE IV

Effects of NAALADase inhibitor and memantine on the development of tolerance to morphine

Treatment / dose mg/kg (N)	Test #1 ED_{50}	Test #2 ED_{50}	Fold Shift
Placebo + Morphine (8)	1.49 ± 0.26	8.85 ± 2.22	6.44 ± 1.17
Placebo + Placebo (8)	2.23 ± 0.42	3.28 ± 0.47*	1.70 ± 0.29*
2-PMPA 30 + Morphine (9)	2.00 ± 0.43	9.47 ± 2.13	5.20 ± 1.26
2-PMPA 50 + Morphine (9)	1.87 ± 0.34	5.41 ± 1.11	3.20 ± 0.66*
2-PMPA 100 + Morphine (10)	1.59 ± 0.30	3.49 ± 0.83*	2.70 ± 0.57*
Memantine 7.5 + Morphine (8)	1.51 ± 0.29	3.52 ± 0.88*	2.60 ± 0.49*
ANOVA: F(5,46) =	0.71; ns	3.891; P<0.01	4.555; P<0.01

[0175] Presented are mean ED_{50} values with ± SEM determined during test #1 (pre-morphine) and test #2 (post-morphine) as well as resulting fold shifts. Asterisks (*) indicate

a statistically significant difference compared to the Placebo+Morphine group that received saline and morphine during the development of morphine tolerance (*p<0.05, Newman Keul's test).

Effects of 2-PMPA on the Tail-flick Response and Antinociceptive Effects of Morphine (Experiment 2)

[0176] Analysis of areas under curve (AUC) revealed that treatment with placebo + 1.5 and 3 mg/kg of morphine produced statistically significantly longer tail-flick responses compared to placebo+placebo treatment. In contrast, 100 mg/kg of 2-PMPA + placebo treatment did not affect tail-flick responses as compared to placebo+placebo treatment. Moreover, this dose of 2-PMPA did not affect antinociceptive effects of 1.5 or 3 mg/kg of morphine (Figure 1).

[0177] Presented in Figure 1 are the time courses of tail-flick responses of mice treated with combination of 2-PMPA and morphine. The N is given in brackets. Inset: Presented are mean ± S.E.M. Area Under Curve (AUC) values calculated on the same data. One way ANOVA $F(5,48)=19.28$, $P<0.0001$ and post-hoc Newman-Keul's test revealed that the treatment with placebo+morphine 1.5 mg/kg and with 100 mg/kg 2-PMPA+morphine 1.5 mg/kg differed significantly (**, $P<0.01$) from placebo+placebo treatment. Similarly, treatment with placebo+morphine 3 mg/kg and that with 100 mg/kg 2-PMPA+morphine 3 mg/kg differed significantly (***, $P<0.001$) from placebo+placebo treatment. Effects of 100 mg/kg of 2-PMPA+placebo treatment did not differ from placebo+placebo treatment. Effects of placebo+respective doses of morphine did not differ from the effects of 2-PMPA+ respective doses of morphine.

[0178] All publications, patents and patent applications identified above are herein incorporated by reference.

[0179] The invention being thus described, it will be apparent to those skilled in the art that the same may be varied in many ways without departing from the spirit and scope of the invention. Such variations are included within the scope of the invention to be claimed.